CAPTIVE MANAGEMENT OF THE HOOK LAKE WOOD BISON RECOVERY PROJECT

PART I: AN OVERVIEW OF MANAGEMENT FOR GENETIC DIVERSITY

Northwest Territories Resources, Wildlife & Economic Development

CAPTIVE MANAGEMENT OF THE HOOK LAKE WOOD BISON RECOVERY PROJECT

PART I: AN OVERVIEW OF MANAGEMENT FOR GENETIC DIVERSITY

G.A. WILSON[1]

K.A. ZITTLAU[2]

and

J.S. NISHI[3]

2003

[1] DEPARTMENT OF MEDICAL GENETICS, UNIVERSITY OF ALBERTA, EDMONTON, AB

[2] DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF ALBERTA, EDMONTON, AB

[3] DEPARTMENT OF RESOURCES, WILDLIFE & ECONOMIC DEVELOPMENT, FORT SMITH, NT

File Report No. 132

ABSTRACT

In this report we review issues in conservation genetics, which pertain directly to genetic management and captive breeding of wildlife. Our goal is to evaluate genetic management options for the Hook Lake Wood Bison Recovery Project (HLWBR), a community-based wildlife conservation project that was initiated in 1996 and is run co-operatively between the Government of the Northwest Territories (NWT), the Aboriginal Wildlife Harvesters' Committee (AWHC) and Deninu Kue' First Nation in Fort Resolution. A principal aim of the project is to salvage genetic diversity from the Hook Lake herd, a wild, free-ranging herd of wood bison in the Slave River Lowlands that is diseased with bovine tuberculosis *(Mycobacterium bovis)* and brucellosis *(Brucella abortus)*. The long-term goal of the co-operative project is to use a captive, disease-free herd to re-establish a healthy herd of free-ranging bison in the Hook Lake area. The current phase of the project, genetic salvage and captive-breeding, is based on a combination of techniques to propagate a healthy captive herd. From 1996 to 1998, a total of 62 calves were captured from the wild Hook Lake herd. At the time of writing (Nov 2002), 57 individuals comprised the founder herd with an additional 84 captive-born animals ranging in age from calves to three-year olds. To date there have been no cases of tuberculosis or brucellosis; all founder animals have been repeatedly tested using a combination of serologic tests for brucellosis and tuberculosis. As the HLWBRP proceeds into a growth phase through captive breeding and total herd size approaches the upper capacity of the facility, it becomes critically important to manage reproduction of the captive herd so as to

minimize the loss of genetic diversity in future generations of captive-born bison. This report represents the first of two parts of an overall genetic management review of the HLWBRP. Here our objectives were to 1) detail the rationale for conservation of genetic diversity, 2) provide an overall framework and rationale for genetic management of captive breeding herds, and 3) explore the various tools available to the conservation geneticist in developing options for breeding management of the recovery project.

TABLE OF CONTENTS

ABSTRACT iii

LIST OF TABLES vii

1. Introduction 1

2. Reasons for maintaining the diversity of the HLWBRP 4
   2.1) Potential negative aspects of low genetic variability at the population level 4
   2.2) Conservation of genetic diversity for reintroduction into the wild 9

3. Measures of genetic diversity 11
   3.1) Factors that affect genetic diversity through time 13
   3.2) Methods to retain genetic diversity 19

4. Captive breeding for genetic diversity 24
   4.1) The phases of a captive breeding program 26
   4.2) Genetic management options 28

5. Minimum viable populations: general background 36
   5.1) Factors that influence MVP for large social mammals 37
   5.2) Population Viability Anaysis (PVA) computer packages 38
   5.3) Conclusions 62

6. Recommendations 62

ACKNOWLEDGEMENTS 68

LITERATURE CITED 69

LIST OF TABLES

Table 1. Current number of bison, Hook Lake Wood Bison Recovery Project, Fort Resolution, NT, April 2002 3

Table 2. Effective size of the wood bison population at Elk Island National Park 18

Table 3. Summary of genetic management options 35

Table 4. Population Viability Analysis (PVA) computer packages 60

104-05119/

1. Introduction

Wood bison were once among the most common ungulates in North America. Their range included northern Alberta and British Columbia, and parts of the Northwest Territories (NWT), Yukon and Alaska (van Zyll de Jong 1986, Guthrie 1990, Stephenson *et al.* 2001). However, their numbers decreased from approximately 100 000 in the year 1800 to about 250 animals by the year 1900, existing mainly in the region of what is now Wood Buffalo National Park (Soper 1941).

Efforts to conserve the wood bison in Canada date back to 1877, with the passing of the Buffalo Protection Act (Hewitt 1921). This was followed by the establishment of Wood Buffalo National Park in 1922 to protect the remaining wood bison, which had increased in numbers to about 1500, all existing in that area (Soper 1941). Unfortunately, 6673 plains bison from Wainwright National Park were shipped to Wood Buffalo National Park from 1925 to 1928 (Ogilvie 1979). Wood bison conservation was irreversibly affected by this action for two reasons: 1) the wood and plains bison hybridized (Polziehn *et al.* 1996, Wilson and Strobeck 1999) and 2) brucellosis and tuberculosis were introduced into the region (Fuller 2002), and have since spread throughout the park and remain endemic to the population (Joly and Messier 2001).

The effect of the hybridization of wood and plains bison in Wood Buffalo National Park on the taxonomic status of wood bison is heavily debated. Some feel that this hybridization has resulted in the loss of wood bison as a subspecies (Geist 1991), while others feel that wood and plains bison are still different

enough to warrant subspecific status (van Zyll de Jong *et al.* 1995, Gates *et al.* 2001). The conservation of wood bison is still imperative[1], regardless of taxonomic status, as the animals in this region have a genetic history that is unique (Wilson and Strobeck 1999, Gates *et al.* 2001).

To date, three attempts have been made to salvage disease-free wood bison from this region (Gates *et al.* 2001, Nishi *et al.* 2002b). The Mackenzie Bison Sanctuary and Elk Island National Park populations were established in the 1960s. Fewer than twenty founders were used for each of these populations. Both the Mackenzie Bison Sanctuary and Elk Island National Park populations contain significantly less genetic variation than their source population, Wood Buffalo National Park[2] (Wilson and Strobeck 1999).

The third salvage attempt was carried out on the Hook Lake herd, located north and west of Wood Buffalo National Park. A total of 62 calves were taken from the region between 1996 and 1998 (Gates *et al.* 1998, Nishi *et al.* 2001, Nishi *et al.* 2002a), of which 57 founders are currently alive (Table 1). A study comparing the success of this genetic salvage operation to the previous salvage attempts revealed that, while the founders of the Hook Lake Wood Bison Recovery Project (HLWBRP) population are more variable than those from

---

[1] In 1978, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) listed the wood bison as "endangered" in Canada (Novakowski 1978). Progress towards recovery (WBRT 1987), resulted in downlisting wood bison to "threatened" status in 1988. Most recently, Ruckstuhl (2000) recommended to COSEWIC to continue to list wood bison as threatened in large part because of the continued threats of bovine diseases, interbreeding with plains bison, and habitat loss, combined with the fact that only two free-ranging disease free herds - the Mackenzie and Yukon herds – meet or exceed the minimum viable population objective of 400 animals.

[2] In 1990, a Federal Environmental Assessment Panel reviewed the issue of diseased bison in Wood Buffalo National Park and recommended that the park bison be depopulated and replaced with healthy animals primarily from Elk Island National Park (Connelly et al. 1990). Had this plan

previous salvage attempts, to date that genetic variation is not well represented in the calves born to the population (Wilson 2001, Wilson *et al.* in prep).

Table 1. Current number of bison, Hook Lake Wood Bison Recovery Project, Fort Resolution, NT, November 2002. Numbers in brackets represent mortalities.

Cohort	Male	Female	Total
Wild-caught founders:			
1996 [a]	5 (1)	13 (1)	18 (2)
1997	4 (0)	16 (0)	20 (0)
1998 [b]	5 (1)	14 (2)	19 (3)
Captive-born:			
1999 [c]	3 (0)	4 (2)	7 (2)
2000 [d]	10 (2)	11 (2)	21 (4)
2001 [e]	11 (2)	11 (2)	22 (4)
2002 [f]	17 (1)	17 (1)	34 (3*)
Total	55 (7)	86 (10)	141 (18)

[a] Female bison reacted on caudal fold test. She and male penmate were killed on 5 March 1997 for post mortem examination (Gates *et al.* 1998).
[b] Female calf euthanized on 15 August 1998 due to severe ataxia after 3.5 months of unsuccessful treatment (culture negative for tuberculosis and brucellosis). Female short yearling died on 12 April 1999 from accidental neck injury.
[c] 1999 cohort mortalities: nutritional myopathy, unknown.
[d] 2000 cohort mortalities: late term abortion (non-disease related), stillborn (hypoxia resulting from dystocia), trauma (kicked in head), unknown.
[e] 2001 cohort mortalities: trauma (exposure), shock, suspected nutritional myopathy (2 calves).
[f] 2002 cohort mortalities: suspected nutritional myopathy (2 calves). *One calf of unconfirmed sex was found dead on its calving date – cause of death was unknown.

As the HLWBRP proceeds through the growth phase and approaches its upper capacity, it is critically important to manage growth and reproduction of the captive herd so as to minimize the loss of genetic diversity in future generations of captive-born bison. This report represents the first of two parts of an overall genetic management review of the HLWBRP. Our objectives were to 1) detail the rationale for conservation of genetic diversity, 2) provide an overall framework

---

been implemented, the genetic diversity unique to wood bison found only in Wood Buffalo National Park would have been lost.

and rationale for genetic management of captive breeding herds, and 3) explore the various tools available to the conservation geneticist in developing options for breeding management of the recovery project.

2. Reasons for maintaining the diversity of the HLWBRP

There are numerous reasons for attempting to maintain the genetic diversity found within the bison of the HLWBRP. These reasons fall into two main categories: the protection of the genetic health of the HLWBRP population, and the conservation of wood bison as a subspecies. The importance of genetic variation to conservation at the population and subspecies level is discussed below.

2.1) Potential negative aspects of low genetic variability at the population level

The genetic health of a population, i.e. its ability to adapt to present or future environmental conditions, rests upon the maintenance of genetic diversity within it. A loss of genetic diversity can have extreme negative effects on the ability of a population to exist through even short periods of time by increasing the effects of inbreeding in a population, and decreasing the population's ability to adapt to different selection pressures.

The genetic load of a population refers to the amount of deleterious recessive alleles in that population. Most mammals have recessive deleterious alleles present in their genome (Wright 1977, Charlesworth and Charlesworth

1987, Ralls *et al.* 1988). These alleles have little to no effect on an individual when present in a heterozygous state. Inbreeding is thought to have a negative effect on the fitness of individuals by increasing the number of loci at which an individual is homozygous for these deleterious alleles. As inbreeding increases, so does the probability that the two alleles an individual has at a locus will be identical by descent (i.e. derived from an ancestor common to both sides of the pedigree), and therefore homozygous (Lacy 1997).

The negative effects of inbreeding on an individual's fitness have long been known. Charles Darwin was among the first to write about a link between the level of inbreeding in domesticated individuals, and the health of these individuals (Darwin 1868). The negative effects of inbreeding include high mortality, reduced competitive ability, greater susceptibility to disease, lower fecundity, and more frequent developmental defects (see for e.g. Wright 1977, Allendorf and Leary 1986, Ralls *et al.* 1988, Hedrick and Kalinowski 2000, Frankham *et al.* 2002). However, it is only recently that the negative effects of inbreeding have been documented in wild mammalian populations.

The most commonly cited examples of inbreeding depression affecting mammalian populations involve various species of big cats. Florida panthers underwent a population bottleneck of about 30 animals, and thus have low levels of genetic variation. These animals have poor sperm quality and often suffer from high levels of microbial parasites (Roelke *et al.* 1993, O'Brien 1994). Lions in the Ngorongoro Crater, Tanzania, reduced to around ten animals in 1962, are less variable and have more sperm abnormalities than nearby lion populations

(Packer *et al.* 1991). Evidence for inbreeding depression has been found in an ungulate population where researchers observed that horn growth is faster in bighorn sheep that have higher levels of heterozygosity (Fitzsimmons *et al.* 1995). As horns are used during mate competition, faster growing horns could result in an increase in fitness.

There is also some evidence that inbreeding depression can negatively affect fitness in bison. Van Vuren (1984) hypothesized about a link between supernumerary teeth and inbreeding. The plains bison population in Badlands National Park, South Dakota, is currently derived from two lineages: the original Nebraska line (NL), and a more recently introduced Colorado line (CL) (Berger and Cunningham 1994). The Colorado line was derived from only three individuals, and was likely less genetically variable than the Nebraska line. None of the five CL bulls were observed to successfully mate during a five-year study by Berger and Cunningham (1994). Also, NL-CL hybrids had reduced growth and delayed onset of puberty over pure NL animals. The authors hypothesized that these results could be attributed to inbreeding effects in the Colorado line. However, it should be noted that Wilson *et al.* (2002) found no relationship between heterozygosity and either weight or reproductive success in a study of wood bison at Elk Island National Park.

The above examples show the relationship between inbreeding and individual fitness. However, in order for conservation efforts to be negatively affected by low levels of genetic diversity, it must be shown that populations with low genetic diversity are less fit, and therefore more likely to go extinct, than

populations with higher levels of diversity. Theory suggests that, if individual fitness is reduced due to the effects of inbreeding, a population's potential for growth will decrease. If this is the case, inbreeding will become more prevalent in the population, and it will enter into an extinction vortex (Gilpin and Soulé 1986, Goodman 1987). In a study of laboratory-raised *Drosophila*, Bijlmsa *et al.* (2000) found that there is a strong correlation between the degree of inbreeding in *Drosophila* populations, and the short-term probability of the extinction of those populations. It is important to note that the negative effects of inbreeding were maintained during their study for over 50 generations. This suggests that genetically depauperate populations in the wild that currently do not seem to be negatively affected by inbreeding; for example, the northern elephant seal (Hoelzel *et al.* 1993), the Swedish beaver (Ellegren *et al.* 1993), and some bison populations (Wilson and Strobeck 1999) are not necessarily safe from these effects should future environmental changes occur.

Berger (1990) discovered that the persistence of bighorn sheep populations was directly affected by the size of those populations, with populations smaller than 50 being more likely to go extinct in 50 years or less. He felt this was due to the levels of genetic diversity in these populations. Saccheri *et al.* (1998) were the first to establish a direct link between levels of genetic variation and extinction in natural populations. They examined a metapopulation of Glanville fritillary butterflies, which occupy alpine meadows. They found a high correlation between the extinction of local populations and their relative levels of genetic diversity, even after allowing for environmental, ecological, and

demographic differences between areas. Therefore, genetic diversity can affect the survival of populations, irrespective of other demographic variables.

Some have suggested that an increased level of inbreeding will not negatively affect the long-term survivability of populations, since the genetic load will be purged during the first few generations that a population exists in low numbers (Templeton and Read 1984, Barrett and Charlesworth 1991). Purging is proposed to occur as follows. When a population has a low density, it is likely that deleterious recessive alleles will become homozygous in some individuals due to the level of inbreeding in the population. Individuals homozygous for these deleterious alleles will be less fit, and therefore will be removed from the population. Consequently, the frequency of these deleterious alleles will decrease, and the genetic health of the population will increase.

However, there is as yet little evidence that genetic purging occurs in mammalian populations (Lacy 1997). In fact, if - as is likely the case - the genetic load of a population is due to a large number of slightly deleterious alleles, inbreeding is more likely to result in extinction than purging of the genetic load (Hedrick 1994, Lacy 1997). Cheetahs, which have decreased genetic variation due to ancient population bottlenecks, still suffer inbreeding effects when bred in captivity (O'Brien *et al.* 1985). This would not be the case if their genetic load had been purged as a result of the increase in inbreeding during their last bottleneck. Bijlsma *et al.* (1999) have shown that, even in cases where purging appears to be effective, it only increases a population's fitness in the environment in which the purging occurred. A management program that attempts to purge a

population of deleterious alleles will almost certainly lose neutral variation that may prove to be useful in altered environmental conditions, and may also impose unexpected and harmful side effects (Wang *et al.* 1999).

Low genetic variation may also decrease a population's ability to evolve through the process of natural selection. Natural selection favours individuals who are better adapted to the current environment. Theoretically, a population with high levels of genetic diversity should have a high adaptive potential, as different genetic material in the population may prove to be beneficial in various environments. Natural selection cannot act without the presence of alternate alleles in a population (Robertson 1960, James 1971). Therefore, populations with low genetic diversity will be less able to adapt to changing climatic conditions or food supplies, or the addition of new predators, parasites, competitors, or diseases (Lacy 1987). However, it is difficult to prove that a population extinction event was a result of low genetic diversity and a consequent lack of adaptive potential (Kelly 2001). While the proximate cause of extinction for a population could be a change in environmental conditions, the ultimate cause may have been the inability of the population to adapt, due to its low genetic diversity.

2.2) Conservation of genetic diversity for reintroduction into the wild

When the goal of a captive breeding program is the eventual reintroduction of individuals to the wild, then the animals in captivity should be maintained in such a way that they remain genetically similar to the wild population, i.e. evolutionary change should be limited (Lacy 1987). Otherwise, genetic changes

may occur during captivity that could jeopardize the individuals' ability to exist in the wild (Frankham and Loebel 1992). As such, attempts should be made to minimize the loss of genetic diversity in the HLWBRP population through the process of adaptation to captivity. We note that adaptation cannot begin before the loss of animals from the founder generation. Therefore, conservation of germplasm from the founders may allow any evolutionary change to be reversed in the future through application of reproductive technology.

Selection will exist in captive breeding programs, eliminating alleles that are maladaptive in the captive environment (Lacy 1994). There is also a very high likelihood that managers will inevitably and artificially select for some traits such as tameness during the handling of captive animals (Darwin 1868). Unfortunately, we do not know what adaptations will be required in the future. Any genetic variation lost in captivity may be the variation that is required as populations are reintroduced into the wild. To date, genetic adaptation to captive environments has been described in fish (Swain and Riddell 1990, Johnsson and Abrahams 1991), and plants (Allard 1988). Frankham and Loebel (1992) also found high levels of selection on laboratory populations of *Drosophila* when their food supply was changed, especially during periods of overcrowding. Adaptations to captive conditions are often disadvantageous in the natural environments. Lacy (1994) outlines a mammalian example where this may be the case. Captive antelope often experience fatal trauma in zoos when they run into fences during flight behaviour after hearing a loud noise. Obviously, this behaviour is disadvantageous in captivity, but is selected for in the wild where

predation on antelopes is common. Antelope that do not exhibit this behaviour may then be selected for in captivity, while these same animals would be selected against upon reintroduction to a natural environment. For these reasons, it is important to try to minimize the amount of selection occurring on animals in captivity.

3. Measures of genetic diversity

The two most important measures of genetic diversity - also termed gene diversity (GD) - are allelic diversity and heterozygosity. Allelic diversity refers to the mean number of alleles per locus in a population. This measure reflects a population's long-term ability to adapt. The more alleles that are present in a population, the greater the probability that the population will adapt to unexpected environmental changes. Heterozygosity of a population refers to the proportion of animals that are expected to have two different alleles at a particular locus. Heterozygosity levels reflect the potential for immediate adaptation of a population. The heterozygosity of a population is proportional to the level of inbreeding that has occurred. Individuals with high heterozygosity levels have a lower risk of extinction, as an individual heterozygous for a particular locus will be better able to avoid the effects of recessive deleterious alleles.

Both allelic diversity and heterozygosity can be lost quickly via genetic drift. Genetic drift is the loss of genetic variation due to the random subsampling of alleles through generations. Typically, the smaller the population, the greater

the rate of loss of diversity. When a population bottleneck occurs, allelic diversity is lost more quickly than heterozygosity and rare alleles are lost more readily than common alleles. The number of offspring produced by a particular founder determines allelic diversity in future generations. If a founder only produces one offspring, 50% of the founder genome is lost. The proportion of a founder's genes in the current population is referred to as gene retention. If gene retention can be maximized through management, the genetic diversity of a population can be maintained. In 1989, Lacy introduced the concept of founder genome equivalents (f_g) to account for the effects that skewed founder contribution and loss of allelic diversity due to drift can have on a population's genetic diversity. Thus, f_g will be lower than the expected gene retention. f_g is simply the number of founders required to obtain current levels of genetic diversity if founder contribution was not skewed and if alleles were not lost by genetic drift. It is calculated as:

$$f_g = 1 / \Sigma (p_i^2/r_i) \qquad (1)$$

where p_i is the founder contribution of the founder 'i' to the population, and r_i is the founder's retention in the population. Current founders are not included in this calculation.

Management approaches for preserving or recovering small populations must focus on the retention of genetic diversity. Although genetic diversity levels will be affected by reductions in population size, rapid selection processes can also reduce diversity, so management efforts should attempt to preserve the population's original genetic state, rather than simply trying to increase the population size. When a captive breeding program is initiated, it is often more

important to maximize gene retention than to prevent inbreeding. Management should be directed toward preserving heterozygosity and allelic diversity. However, maximizing for one measure does not necessarily ensure the maximization of the other. Most genetic management methods are designed to maximize the heterozygosity in a population. However, by equalizing founder contribution within a population, allelic diversity can be maintained as well.

3.1) Factors that affect genetic diversity through time

Genetic diversity can be lost from a population through the processes of genetic drift and selection. As an example, assume that there are three alleles at a locus in a population of ten individuals. If one of the alleles is only found in a single individual, and this individual fails to breed during its lifetime, then that allele is lost through the process of genetic drift and the genetic diversity in the population decreases. Random sampling of a small number of genes results in greater fluctuations in their frequencies than sampling a large number of genes. Consequently, as population size decreases, the effect of genetic drift on the diversity of a population increases, as does the amount of inbreeding in that population. The loss of heterozygosity expected in a population through time can be calculated with the formula:

$$H_t = H_0 * (1 - (1/2\ N_e))^t \qquad (2)$$

where H_t is heterozygosity at time t, H_0 is heterozygosity at time 0, and N_e is the effective population size. This formula illustrates the inverse relationship between the effective population size, and loss of heterozygosity over generations.

The concept of an effective population size (N_e) was introduced by Wright (1931). N_e is the size of an ideal population, with random union of gametes between generations, which loses genetic variation at the same rate as the population of interest. The more the population of interest differs from an ideal population, the greater the difference between N (actual size of the population) and N_e. Most of the factors that dictate the difference between N and N_e are demographic in nature. These include fluctuating population size, variance in reproductive success, and sex ratio bias (Wright 1969, Crow and Kimura 1970).

The effective size of a population that undergoes fluctuations in population size can be estimated with the formula:

$$N_e = n / \sum(1/N_i) \qquad (3)$$

(Lande and Barrowclough 1987) where n is the number of generations, and N_i is the size of the population in each generation. This is a geometric mean, which means that small values of N_i have a large effect on the final value of N_e. N_e is maximized when variance in N_i is low. Therefore, populations that stay at a relatively constant size will have larger values of N_e, and will not lose genetic diversity as quickly as populations that vary greatly in size. Also, the amount of genetic diversity lost due to a population bottleneck is greatly affected by the length of the bottleneck.

If one of the genders in a population has differential reproductive success, the ratio of effective size to actual size of that gender can be estimated with the formula:

$$N_e/N = k / (k-1+V_k/k) \qquad (4)$$

(Rockwell and Barrowclough 1995, modified from Crow and Kimura 1970), where N is the number of individuals of a specific gender, k is the mean lifetime reproductive success, and V_k is the variance in lifetime reproductive success. This formula assumes the population size is relatively stable, and much larger than two. N_e is maximized when the variance in reproductive success is low. Populations in which each family produces the same number of offspring will have higher values of N_e, and therefore will lose genetic variation at a lower rate than those in which variance in reproductive success is high. This formula shows that, in polygamous species where variance in reproductive success is the norm, the effective size of the population will be much lower than the actual size of the population. Historically, most captive breeding programs for herd animals use few males as sires, reducing the effective size of these populations (Ryder and Fleischer 1996). Wilson *et al.* (2002) have shown that variance in reproductive success occurs in both male and female bison, although it is much greater in males. However, this study only looked at reproductive success over four years. It is possible that variance in reproductive success is lower when taken over the lifetime of the individual. Regardless, this should be taken into account when calculating N_e for bison.

N_e can be estimated in cases where there is sex ratio bias with the formula:

$$N_e = 4N_mN_f / (N_m + N_f) \quad (5)$$

(Wright 1931), where N_m and N_f are the number of males and females in the population, respectively. This formula assumes random mating, discrete

generations and constant population size. Any deviation from these assumptions will result in a further increase in N_e (Vucetich and Waite 1998). It should be noted that only reproductively successful individuals should be included in the counts for each gender. Populations that contain an equal number of reproductively successful males and females will have a larger effective population size, and will therefore lose genetic variation slower than those with greater sex ratio bias. Kelly (2001) recommends calculating the effective size of each gender with formula (4), allowing for variance in reproductive success, and then using those values as the N_m and N_f in formula (5). Only present-generation breeders should be included in all N_e calculations (Lacy 1995).

The discrepancy between the effective size and actual size of a population is often very large. In studies of *Drosophila*, Briscoe *et al.* (1992) estimated the ratio of N_e/N to be in the range of 0.004 - 0.051. In many captive populations, N_e/N is between 0.1-0.5 (Lacy 1995). In a natural population of cheetahs, N_e/N was estimated to be 0.15 (Kelly 2001). In this population, variance in reproductive lifetime success was the variable that resulted in the largest decrease of N_e/N. The plains bison population at Badlands National Park has an N_e/N between 0.28 and 0.45 (Berger and Cunningham 1994).

Because of the wealth of information available about the structure of the wood bison population at Elk Island National Park, including the reproductive success of males and females (Wilson *et al.* 2002), N_e/N can be estimated for this population. Since the Wilson *et al.* (2002) study only spanned four years, variance for lifetime reproductive success cannot be directly measured. However,

if mortality is not age-dependent and the variance in reproductive success is limited to prime age classes, it can be estimated by examination of the variance in success of the prime aged individuals (Berger and Cunningham 1994). Variance in the number of calves sired by males who were of prime breeding age over the course of the study was estimated as 16.3. Formula (4) could not be used to calculate N_e from this, as we do not know the mean reproductive success. However, a formula derived by Crow and Kimura (1970) does not require this value:

$$N_e = (4N - 2) / (V_k + 2) \quad (6)$$

This could then be used to calculate the effective number of males in Elk Island National Park, given the number of males of breeding age in each year of the study. A similar calculation was not performed for the females, as variance in female reproductive success was low. Formula (5) was then used to obtain values of N_e each year, given the number of males and females in the population (Table 2). N_e/N was approximately 1/3 during each year of the study. This means that in order to obtain a population of a specific effective size, roughly three-fold that number of breeding animals must be used. Also, N_e is solely based on animals of breeding age, and does not include pre- or post-reproductive animals.

As a result of the discrepancies between N_e and N in most studied populations, it is important to determine the effective size of populations before attempting to predict the loss of diversity through time. Estimates using actual population sizes will be overly optimistic.

Table 2. Effective size of the wood bison population at Elk Island National Park

	1996	1997	1998	1999
N_m	72	78	87	100
N_f	116	122	129	126
N	188	200	216	226
N_{em}	15.6	16.9	18.9	21.7
N_e	55.0	59.4	65.9	74.1
N_e/N	0.29	0.30	0.30	0.33

N_m: number of breeding age males in the population
N_f: number of breeding age females in the population
N: number of breeding age individuals in the population
N_{em}: effective population size of breeding males
N_e: effective size of the population

Another problem exists for populations with low effective population size. The ability of selection to eliminate deleterious alleles from the population is inversely proportional to the effective size of that population. Selection can only eliminate deleterious alleles when $s>1/2N_e$, where s is the strength of selection pressure (Kimura 1983). Therefore, it is possible for deleterious alleles to become fixed in a population through the process of genetic drift if the population is so small that the allele acts as if it were neutral (Lande 1994, Lynch and Gabriel 1990, Hedrick and Kalinowski 2000). This could result in a decrease in the fitness and survival rates of the population through time. This decrease in fitness may be an even greater threat in captive management, as it would not likely be noticed until the population is reintroduced to the wild, where animals are not cared for (Lynch 1996). Lacy (1994) outlines a case where a potentially deleterious allele is increasing in a recently established captive population. A translocation of the Y chromosome onto an autosome was found on one of six

male golden-headed lion tamarins that have been used in breeding programs in the USA. This male has sired ten of 52 animals born in captivity.

Lande (1994) predicted that populations with N_e as large as 1000 would be affected by the fixation of deleterious alleles, while Lynch *et al.* (1995) felt that a more reasonable value was N_e=100. However, in a laboratory study of *Drosophila melanogaster*, Gilligan *et al.* (1997) found no evidence for a decrease in fitness due to an accumulation of deleterious mutations over a span of 45-50 generations, for N_e values as low as 25. Also, the genetic load of their captive population (which would increase as the number of fixed deleterious mutations increased) did not seem to be any different from the wild population after their experiment. Therefore, the accumulation of deleterious alleles may not be a significant problem in captive populations.

3.2) Methods to retain genetic diversity

One of the first steps in determining the best method for ensuring that the loss of genetic diversity is minimized, is to determine the ultimate goal of the conservation program. Franklin (1980) derived one of the most commonly cited goals of genetic management. He suggested that, in order to ensure that new mutations restore heterozygosity at the same rate that variation is lost through the process of genetic drift, a minimum N_e of 500 should be used. If, instead, the short-term goal of the breeding program was to minimize the deleterious effects of inbreeding, the minimum required N_e could be lowered to 50. This has become known as the 50/500 rule.

Another common goal in the managernent of captive populations is to minimize all evolutionary change, including the addition of new mutations to the population. Soulé *et al.* (1986) recommended that a reasonable goal was to retain a minimum of 90% of the genetic variation within a population for a period of 200 years. Their feeling was that new technologies would exist for maintaining and regenerating species after this time. Many conservation plans base their decisions on meeting this goal. The actual effective population size required to retain 90% of the diversity for 200 years is species-dependent. However, rather than unquestioningly accepting these somewhat arbitrary goals that specify persistence of a population over a specified time period, it is more important to recognize that persistence and genetic management objectives be considered and evaluated over a range of time and geographic scales (Allendorf & Ryman 2002). In addition, genetic goals in management of captive populations necessitate a compromise with available resources that will determine carrying capacity of breeding facilities (Frankham *et al.* 2002).

Some of the most obvious methods of minimizing the loss of genetic diversity through time as a result of genetic drift are to maximize the N_e/N ratio (as discussed in Section 3.1). For example, efforts should be made to eliminate fluctuations in population size. If a population is reduced in size, it should be rapidly brought back up to the carrying capacity, as the length of the bottleneck greatly reduces N_e. Minimizing the sex ratio bias will also maximize N_e/N. In polygynous species such as bison, a 50:50 sex ratio may not be appropriate, as most of the breeding will only be done by a few of the bulls (Wilson *et al.* 2002).

Any individual who does not breed should not be considered in calculations of effective population size. Therefore, keeping the population at a 50:50 sex ratio would only result in an increase in the number of nonbreeding males in the population that would be taking resources away from other individuals. However, while a 50:50 sex ratio may not be feasible in polygamous species, the effect of sex ratio bias on the effective size of the population should be considered when calculating loss of diversity.

Managers should also make attempts to limit the adaptation of a population to its captive environment. Most methods for minimizing adaptation will also decrease the amount of genetic drift in a population. In fact, they are inextricably linked. For this reason, although we focus on methods to minimize adaptation to captivity, we also discuss the effects of genetic drift where appropriate. Frankham and Loebel (1992) list a number of steps to minimize adaptation to captive environments:

1) Continued introduction of genes from the wild will slow the rate of adaptation in proportion to the contribution of wild genes. This will not only decrease the chance that a population will become adapted to its local environment, it will also act to slow genetic drift. Wright (1931) showed that one migrant per generation will reduce the likelihood that a locus will become monomorphic, and five migrants per generation will virtually halt genetic drift. Genetic migrants have been theoretically shown to have a great effect on the diversity of a population (Lacy 1987). However, care must be taken to ensure that the

migrants are appropriate, i.e., that they are not from a genetically dissimilar population (Ryder and Fleischer 1996). The history of bison conservation contains many examples of the inappropriate mixing of groups through the exchange of animals. These include the addition of plains bison to Wood Buffalo National Park, and the movement of plains bison - now known to have hybridized with cattle - from Custer State Park, South Dakota, to many other bison populations (Polziehn *et al.* 1995, Ward *et al.* 1999). Any influx of genes from the addition of migrants is irreversible after only a few generations.

2) Reduce the selection for adaptive genes by equalizing family sizes. If family sizes are kept equal, then the competition between families is reduced, and adaptive alleles cannot spread throughout the population (replacing all other alleles). An equalization of family size should halve the rate of genetic adaptation. Equalizing family size will also reduce the variance in reproductive success, thereby increasing effective size of the population.

3) The rate of adaptation to captivity is inversely proportional to generation length. This can be manipulated by increasing the age at first reproduction, and the length of time between mating events. Frankham and Loebel (1992) suggest breeding animals when young to ensure that all animals get a chance to breed before they are removed from the population, but keeping their youngest offspring as the next generation. Since diversity is lost from populations between generations (see formula (2)), an increase in generation

length would also minimize loss of variation through time. Lowering the mortality rate would have an equivalent effect. As an extreme example, if the founders are still present in a population, then no variation has been lost. For this reason, cryopreservation of embryos and sperm should be undertaken, if possible (Frankham and Loebel 1992).

4) Genetic adaptation to captivity will be slow when the captive environment is similar to the wild environment. Therefore, practices such as supplemental feeding, inoculating against diseases, veterinary care, and the maintenance of group structures different from those found in wild populations will all increase adaptation to the captive environment.

Another method for reducing the amount of diversity lost as a result of genetic drift is to subdivide captive populations. Subdivided populations rapidly lose genetic variation from within each subpopulation, but retain variation across the subpopulations better than a single randomly breeding population (Lacy 1987). If these subpopulations are dispersed in different environments, then different alleles should be selected for in each region. This would minimize the effect of adaptation to the captive environment in the entire population (Lacy 1994). However, while the effects of inbreeding on small populations occur quite rapidly, the benefits of a subdivided population occur more in the long term (beyond ten to twenty generations). Therefore, the number of generations that the population will remain in captivity before being reintroduced into the wild will dictate the effectiveness of keeping the population subdivided (Lacy 1987).

4. Captive breeding for genetic diversity

The long-term management goal for a small captive population should be the preservation of genetic diversity. The genetic composition of each captive population should be as similar as possible to the wild population. This should be done through the retention of founders' genetic diversity and the maintenance of a stable population in accordance with habitat carrying capacity. From a genetic standpoint, management efforts should aim to prevent inbreeding depression and the loss of genetic diversity. To maintain genetic diversity in a captive population, it is necessary to obtain a sufficient number of founders to accurately represent the heterozygosity and allelic diversity of the population. The maintenance of allelic diversity will require a greater number of founders than the maintenance of heterozygosity.

Breeding strategies for the maintenance of gene diversity in a population are often based on the assumption that each founder has an equal genetic value. In reality, however, this is not the case. Each population has adapted to the environment in which they live, and it is likely that certain alleles are rare because selection has not favoured them. However, geneticists and managers should not assess which alleles are beneficial and which are deleterious for the population, as the fitness value of these alleles in future environments is unknown.

Bryant and Reed (1999) suggest that populations be managed so alleles of less adapted individuals are lost from the population. However, this will result in a loss of genetic diversity at selected and unselected loci alike, as well as

adaptive diversity, and may eventually lead to inbreeding effects. Conservation programs typically accept an increase in inbreeding by about 1% per generation, and a cumulative increase in inbreeding of approximately 10% (Lacy 2000). The management goal of most captive breeding programs is to retain 90% of the "wild" genetic diversity for 200 years (Soulé *et al.* 1986). But, the actual target number should be dependent on the effective size of the founders, the growth rate and generation time. Population viability is impacted by the interactions between population size and genetic diversity. As population size decreases, the rate of genetic drift increases, as do the effects of inbreeding. Consequently, small captive populations require intensive genetic management.

Management for the retention of genetic diversity requires knowledge of five components: 1) the number of founders for a population, 2) their genetic contributions to all individuals in the population, 3) the relationships among individuals within the population, 4) the genetic importance of each individual and 5) the effective population size. These factors can be determined as follows: The number of founders in a current population can be determined with the construction of a pedigree. Founders are identified as any individual that has no ancestors currently living in the population. Their genetic contribution can be determined by calculating the percentage of each individual's genes that came from each founder. This is then averaged over the whole population. The amount of genetic diversity that has been lost from the founders due to genetic drift and population bottlenecks can be measured using computer simulations and "gene drop" analysis (see Section 4.2, page 33).

Relationships within the population can be determined by measuring inbreeding coefficients and kinship coefficients. These coefficients can be calculated using GENES pedigree analysis software (Lacy 1990) or PM2000 software (Pollak *et al.* 2002) and assist with establishing reproductive strategies. The genetic importance of each individual can be measured in a number of ways (see Section 4.2). Individuals with high genetic importance should then be given breeding priority. High priority breeders should only be bred with other high priority breeders to avoid the intermingling of high and low priority alleles.

This information can then be used to estimate the effective population size, which will be useful for determining the extent that the population must be managed (see PVA/MVP Section 5). Furthermore, this information will give an indication of how well the population is able to retain the genetic diversity that it possesses. That is, a small N_e will lose genetic diversity more quickly than a higher N_e. Also, the average contribution of a founder will affect the retention of genetic diversity in future generations, as will many demographic factors.

4.1) The phases of a captive breeding program

A population that will be used in a captive breeding program will go through three different phases: the founder phase, the growth phase, and the capacity phase (Lacy 1994). It is important for the manager to be aware of the current phase of his population, as different types of management may be recommended for these three phases in order to minimize the long term loss of diversity.

1) The Founder Phase

This is the initiation of the project. During this phase, animals taken from the wild are used as the founders for the captive population. These founders should be as genetically similar to the animals found in the wild population as possible, i.e. the founder should contain the same alleles as those present in the wild population, in the same frequencies (Lacy 1994). Formula (2) can be used to calculate the heterozygosity expected in the founding generation, based on the number of animals in this generation, by setting t=1. It is worth noting that the N in this formula is based on the effective number of individuals. If the founders are captured from the same region, N_e may be lower than N, as the sample of founders may contain close relatives. Also, any founder who does not breed should not be included in calculations of genetic diversity in the population. N_e will also be affected by deviations from a 50:50 sex ratio (formula 5).

2) The Growth Phase

During this time, a population increases in size until carrying capacity is reached. As shown in formula (3), genetic diversity can be quickly lost from the population during bottlenecks. The growth phase of a captive population can be thought of as such a bottleneck. Therefore, it is important that the population is brought up to carrying capacity as quickly as possible. A low rate of growth during this phase will increase the likelihood of inbreeding depression (Moehlman *et al.* 1996). However, care should be taken to ensure that each of the founders

reproduces in approximately equal amounts, as otherwise the diversity represented within less successful founders could be lost. It is undesirable to remove animals during the growth phase, unless they are replaced by more genetically valuable animals that would otherwise be excluded (Lacy 1994). The HLWBRP population is currently in this stage. To date, only four of the 12 males in the population have been reproductively successful. To ensure that the genetic diversity represented in the unsuccessful males is not lost, efforts should be made to allow them to reproduce.

3) The Capacity Phase

In this phase, the population is maintained at an approximately stable size for the remaining length of the program. Most of the methods to minimize loss of diversity described in other sections are intended to be applied to this phase. While any alleles lost from the population in prior phases cannot be replaced, this phase can be used to equalize any disparities in founder allele frequencies by preferentially breeding the animals most likely to possess rare alleles (Lacy 1994). Methods for determining the presence of rare alleles in individuals are outlined in Section 4.2.

4.2) Genetic management options

Several genetic management strategies have been developed for ensuring the maintenance of genetic diversity in a small population through time. The two primary concerns of genetic management are avoiding inbreeding

depression and the loss of alleles due to genetic drift (see Section 3.2). In order to do this, the number of founders in a population must be maximized and breeding should be equalized so that alleles are not lost through genetic drift. The most common strategies are discussed below (see Table 3):

Maximum Avoidance of Inbreeding (MAI)

Kimura and Crow (1963) showed that the loss of heterozygosity could be minimized through the maximum avoidance of inbreeding. This strategy recommends a system in which mating occurs between the least related individuals (Figure 1 from Kimura and Crow 1963). It starts in the current generation, assuming all individuals are equally related. This system is useful for decreasing the loss of heterozygosity at a rate dependent on the effective population size. Naturally, this loss will be a concern for small, captive populations that are unlikely to have a large N_e. MAI has no effect on minimizing loss of allelic diversity through drift. To minimize gene frequency drift, Kimura and Crow (1963) suggest that the number of progeny be kept constant for each generation and that the population be subdivided into as many lines as possible to reduce the variance in allele frequencies. Subdividing the population slows the loss of alleles through each generation.

This procedure is the simplest of the genetic management strategies as, unlike all other measures, no prior knowledge of the pedigree is required. This strategy minimizes further inbreeding in a population, but does not account for the number of founders or their genetic representation within the population. As

pedigree information is not used for this procedure, inbreeding may not always be avoided.

Mean Kinship (MK)

Mean kinship aims to minimize the overall relatedness within a population, thereby maintaining the genetic diversity. The MK method has been shown to be more effective at retaining genetic diversity (heterozygosity) than MAI, Founder Importance Coefficient (FIC) (see below), and random mating (Montgomery *et al.*1997, Ballou and Lacy 1995). Montgomery *et al.* (1997) showed that, in Drosophila, MK had no significant effect on reproductive fitness even though inbreeding was significantly reduced.

An MK breeding program is set up by first calculating a Mean Kinship (MK) value for each individual (Ballou and Lacy 1995). MK is a measure of the individual's genetic importance. It is the average probability that two alleles from randomly selected individuals are identical by descent. MK is calculated as:

$$MK_i = (\Sigma\ f_{ij}) / N \qquad (7)$$

where f_{ij} is the kinship coefficient and N is the number of individuals in the population. Genetic diversity (heterozygosity) of the population can be calculated as 1-MK. Therefore, by minimizing MK, genetic diversity is maximized. This value can be calculated using GENES pedigree analysis software (Lacy 1990) or PM2000 software (Pollak *et al.* 2002). After MK values are calculated, population founders are then ranked by their genetic importance (lowest MK value suggests highest importance) and breeding can be managed accordingly. This method is useful in equalizing founder contributions.

Minimizing kinship does not account for linkage, recombination, or mutation, which could influence the genetic diversity. Also, this method does not account for age-structure in a population; MK values are calculated from the total gene pool. Thus, it can present problems when a low MK value (i.e. high genetic importance) is calculated for an individual that is past reproductive age. Such an individual would be given breeding priority over other individuals, when in fact it can no longer breed. A potential problem with breeding for low MK is that it doesn't take age into account. Also, it is possible that diversity will be lost when individuals with low (but not lowest) MK are not chosen to be bred until they are past reproductive age. This can be overcome by weighing MK by the age of all individuals in the population.

Another possibility is to use kinship value (KV), which measures MK under demographic constraints. The expected heterozygosity of the descendant population can be determined by measuring its KV. KV is the mean of MK values between an individual and all other individuals in the population, weighted by the reproductive value of each age class (Ballou and Lacy 1995). MK and KV will be the same for species that have long generation times. This measure is useful for estimating the retention of genetic diversity in the next generation if the population is bred randomly but reproduces according to its own life table expectations. As MK and KV are calculated relative to all the individuals in a population, the addition or loss of animals (i.e. through the processes of birth and death) will alter these values. MK and KV should be recalculated when the composition of the population is altered.

Founder Importance Coefficient (FIC)

The Founder Importance Coefficient (FIC) is another measure of the genetic importance of an individual. FIC is calculated as the average founder contribution within each individual, weighted by the founder contribution to the entire population. Individuals can be ranked according to their genetic importance and those with low founder contribution (i.e. greater genetic importance) should be given breeding priority (see MK). It is important to note that in order to equalize the distribution of founder genes in the population, individuals with low FIC should be bred with other individuals of low FIC.

FIC does not account for the effect that founder contribution has on an individual's genetic importance. Consequently, breeding priorities can be misidentified. Also, this method cannot discriminate between siblings, which will have the same founder contribution. This could lead to inbreeding in some cases (eg. If two low FIC individuals, which resulted from the same mating, are bred together). This method is less effective at retaining genetic diversity than MK. For these reasons, FIC is not frequently used to estimate genetic importance and to subsequently recommend breeding strategies (Ballou and Lacy 1995).

Genetic Uniqueness (GU)

Genetic uniqueness (GU) is the probability that a particular allele carried by an individual is unique (Ballou and Foose 1996). Managing for GU can increase genetic diversity in a population. Again, individuals in a population can

be ranked according to their GU value. If an individual has a high GU, it is assumed to have high genetic importance, and should consequently be given high breeding priority. However, this method does not account for alleles that are rare, but not unique. That is, if an allele has only two copies in a population, it will not be given high genetic importance. This can skew the results and lead to misdirected breeding priority.

Genetic uniqueness can be calculated using "gene drop" analysis. Gene drop analysis is a Monte Carlo simulation that calculates gene survival in the current population. Each founder is assigned two unique alleles, and the transmission of the alleles is followed from generation to generation through simulations.

Mean Profile Similarity (MPS)

Mean Profile Similarity (MPS) has been shown to provide information about founder relatedness and can therefore be used to determine genetic importance of individuals (Haig *et al.* 1994). MPS is based on the analysis of restriction fragment length polymorphisms (RFLPs) of regions of the genome, and is calculated as the proportion of total DNA fragment bands shared between two individuals. This method is not very effective for determining relatedness between individuals (it produces a high variance for estimates of individuals), but it can estimate overall relative relatedness in a population and subsequently, relative genetic importance. Most importantly, MPS can still be calculated if a complete pedigree is not available. However, it requires genotype information for

all individuals. As this method is useful for determining the distribution of genetic importance within a population, it may lead to a better understanding of the N_e for the population. Thus, MPS may reveal information about population structure. MPS has not been further tested for its efficiency.

Marker-Assisted Selection (MAS)

MAS is a genetic management approach that can be used to increase N_e in small populations (Wang 2001). MAS is, however, an impractical method as it requires at least two markers per 100cm of chromosome length, so a large number of markers and a genomic map are required. It also requires a male: female ratio of greater than one. As the selection target is a chromosome, this method may not retain genetic diversity in a population, nor will it have an effect on relatedness or founder contribution.

Table 3. Summary of genetic management options

Option	Objective	Benefits	Limitations
Maximum Avoidance of Inbreeding (MAI)	Minimizes further inbreeding in a population by decreasing the loss of heterozygosity	- MAI is the simplest option. - No prior knowledge of the pedigree is required. - Useful for populations that have a small N_e.	- Does not minimize loss of allelic diversity. - Does not account for the number of founders or their genetic representation within the population.
Mean Kinship (MK)	Minimizes overall relatedness within a population.	- MK is more effective at retaining genetic diversity than MAI, FIC or random mating - Useful for equalising founder contributions. - An age-weighted MK, kinship value (KV), can estimate the retention of genetic diversity in the next generation.	- MK may have no significant effect on reproductive fitness even if inbreeding is reduced. - Does not account for linkage, recombination, or mutation. - Does not account for age-structure in a population (need KV).
Founder Importance Coefficient (FIC)	Measures the genetic importance of an individual based on founder contribution.	- FIC is the only option that calculates founder contribution within each individual. - Can be used to establish breeding priority of individuals.	- Does not account for the effect that founder contribution has on an individual's genetic importance. - Cannot discriminate between siblings, which will have the same founder contribution. This could lead to inbreeding. - Less effective at retaining genetic diversity than MK.
Genetic Uniqueness (GI)	Measures how unique an individual is based on their alleles.	- GU can be used to calculate gene survival in the current population using "gene drop" analysis. - Can be used to establish breeding priority of individuals.	- Does not account for alleles that are rare, but not unique - Requires "gene drop" analysis software.
Mean Profile Similarity (MPS)	Estimates overall founder relatedness in a population and can be used to determine genetic importance of individuals.	- MPS can determine the distribution of genetic importance within a population - May reveal information about population structure. - A complete pedigree is not required. - Can be used to establish breeding priority of individuals.	- Not very effective for determining relatedness between individuals - Requires genotype information for all individuals - MPS has not been tested for its efficiency.
Marker-Assisted Selection (MAS)	Minimizes inbreeding and genetic drift at the average locus.	- MAS can be used to increase N_e in small populations. - More effective than MAI and possibly MK for highly fecund species. - Much less computer demanding than other systems.	- Requires a large number of markers and a genomic map. - Requires a male: female ratio of greater than one. - May not retain genetic diversity in a population. - Will not have an effect on relatedness or founder contribution.

5. Minimum viable populations: general background

Estimates of minimum viable population (MVP) sizes are used to infer the minimum number of individuals required for a population to have a certain probability of persistence for a given length of time. Early estimates of MVP were based solely on the demographics of the population (MacArthur and Wilson 1967, Richter-Dyn and Goel 1972, Leigh 1975). These studies illustrated that once populations were reduced to a certain size, they quickly became extinct. Later MVP estimates were based upon the amount of genetic diversity found within populations (for review, see Frankel and Soulé 1981, Beissinger and McCullough 2002). However, MVP is not solely affected by demographic and genetic factors. Shaffer (1981) identified four main factors that should be taken into account when performing MVP analyses: demographic stochasticity, genetic stochasticity, environmental stochasticity, and catastrophes (described in Section 5.1). Nunney and Cambell (1993) believe that these effects should be considered when determining MVPs. The effect that these factors have on different taxa depends on their specific ecologies and life-history traits, and hence no universal estimate or application of MVP exists. It should also be noted that MVP analyses describe the minimum effective size (N_e) of a population (the relationship between actual and effective population size is described in Section 3.1). MVP analyses should be considered a lower bound, and not a specific population size to aim for.

An analysis of MVP is typically conducted within the framework of a population viability analysis (PVA). Population viability analysis is a concept and a tool that has been used extensively by conservation biologists to create theory, analyse data, project population trends and make policy and management decisions (Boyce 1992, Beissinger 2002, and see Caughley 1994). A number of programs are currently available for conducting PVAs and estimating MVPs (see Section 5.2). PVAs are useful tools but should not be used without a clear understanding of their limitations and inherent assumptions (Brook *et al.* 2000, Coulson *et al.* 2001, Brook *et al.* 2002, Ellner *et al.* 2002). PVAs should not be relied on solely to assess risk to populations. Instead, PVAs are more helpful in identifying potential factors limiting a species' persistence (Boyce 1992, Caughley and Gunn 1996, Beissinger and McCullough 2002). PVAs provide a useful conceptual framework, but empirical data should also be collected and applied in order to test and verify the recovery and ecological underpinnings of species declines. PVAs have recently been performed for a number of endangered species (see for eg. Maguire *et al.* 1995, Gaona *et al.* 1998).

5.1) Factors that influence MVP for large social mammals

Although the importance of genetic factors affecting population persistence, and hence MVP analyses, should not be understated, they are detailed in Section 3.1. As such it will not be discussed here.

MVP analyses also consider demographic parameters in evaluating population persistence. Demographic stochasticity is the chance occurrence of

events internal to a population that affect its demographic makeup, and can come in the form of random variation in sex ratio, birth rates, or death rates. A critical result of demographic stochasticity is the Allee effect (Allee *et al.* 1949). Allee *et al.* (1949) describe the idea that, once population density decreases to a certain level, it cannot increase and the population is doomed to become extinct. The Allee effect can be a result of the inability to find a mate, difficulty in fending off predators or competitors, or inbreeding depression (Boyce 1992).

Environmental stochasticity is attributable to variation in the ecosystem in which a population lives. This variation can come as changes to quality and quantity of habitat, environmental change, and biological factors such as a change in the relationship between a population and its predators, competitors, parasites or diseases (Boyce 1992). Environmental stochasticity is unpredictable and highly variable, making it difficult to model in MVP analyses.

In most captive populations, demographic and environmental stochasticity can be controlled, or decreased to negligible levels. The elimination of environmental stochasticity has been shown to lower MVP size ten-fold (Shaffer 1987). Allee effects should not be an issue in captive populations, which will again cut MVP by 50% (Nunney and Campbell 1993). As a result, genetic stochasticity has the largest effect on the MVP analyses of captive populations.

5.2) Population viability anaysis (PVA) computer packages

PVA packages are useful for modeling the complex, interacting processes (both deterministic and random) that affect the viability of populations (Beissinger

and McCullough 2002). A number of different computer simulation programs have been created. Each program makes different assumptions and may vary with respect to the importance that certain factors are given, and even the inclusion of certain factors that affect PVA. If certain factors are not incorporated into a model, other factors may be influenced. The outcome of a program will differ based on the user's understanding of the functions included in the package, and the user's biases (Brook *et al.* 1999). Consequently, the projections of each package often differ significantly (Lindenmayer 1995, Brook *et al.* 1997, Brook *et al.* 1999). One of the most common factors that can lead to significant differences between programs is the effect of stochastic variation in breeding structure (Brook *et al.* 1999). Also, for many programs, the species of interest and its life-history traits have a large effect on the results obtained (Mills *et al.* 1996, Brook *et al.* 1999). Most packages differ with regards to their target projections; some track changes in individuals (GAPPS, VORTEX), whereas others track cohorts within metapopulations (INMAT, RAMAS).

Although almost all programs have been used for endangered species management and conservation (see Brook *et al.* 1999, Brook, *et al.* 2000, Coulson *et al.* 2001, Brook *et al.* 2002, Ellner *et al.* 2002), the choice of PVA package will have a large impact on management decisions. In the past, most population viability studies have indiscriminately used VORTEX to estimate extinction rates (Asquith 2001). However, based on the aim of the study and the information available, this program may not provide accurate results. The selection of computer program should be based on two main criteria: the

objective of the study (i.e. genetic vs. demographic) and how the strengths, limitations and assumptions of the particular program match the available data for the population in question. If the criteria for selecting an appropriate PVA package cannot be met, it is recommended that a population-specific program be created for the question at hand. For instance, species with complex life-history traits, such as those that reproduce sporadically (i.e. in response to climate) or with multi-annual population cycles should not be modeled with these programs.

In the following section, we describe the goals, strengths, limitations and assumptions of the most commonly used PVA computer simulation programs. We discuss the pros and cons of these programs from our perspective of prospective use to the HLWBRP. We summarize the salient points of this discussion in Table 4.

VORTEX (Lacy and Kreeger 1992, Lacy 1993, Miller and Lacy 1995, Lacy *et al.* 2003)

VORTEX was developed to examine endangered species populations for establishing conservation strategies. The objectives of the program are to determine how species survival is affected by the interaction between genetic factors and population demography. VORTEX can estimate the probability of extinction, the time to extinction, and changes in population size and genetic variation over time. It is most useful for mammals, birds, and reptiles that have low fecundity and long life spans.

Strengths:

Probably the most important strength of VORTEX is that it is one of the few PVA packages that can model the effects of genetic changes in a population. Also important is that VORTEX is an individual-based program and can therefore track the fate of individuals rather than simply modeling the overall viability of cohorts. Also, VORTEX can incorporate details on behaviour and mating systems, and allows the user to supplement or harvest the population if desired. Although Lindenmayer *et al.* (1995) did not consider VORTEX to be a user-friendly program, we have found that the software is very easy to understand and use.

Limitations:

Partial correlations between environmental variation and birth rates, death rates and carrying capacity cannot be modeled using VORTEX. Furthermore, this program is also not useful for populations that are strongly affected by interactions with species that have complex dynamics, such as predator-prey interactions. One of the most important limitations of the program is that a maximum of only 20 populations can be examined, and the population sizes should not exceed 1000 individuals. Consequently, VORTEX should not be used for highly fecund populations. Although VORTEX is the only PVA package that can model changes in genetic variation, it may provide a low estimate of the rate of loss of genetic diversity. The model assumes an equal probability of breeding, but because this rarely occurs in natural populations, the actual loss of genetic variation will occur more rapidly than estimated. Overall, Lindenmayer *et al.*

(1995) declared VORTEX to be non user-friendly, but this is likely because the program is designed for use with partial assistance from the architect (R. Lacy). The program also has heavy data requirements, and in most situations many of the parameters will be unknown.

Assumptions:

Reproduction: All individuals of reproductive age have an equal probability of breeding. Birth and death rates are constant from the age when the animal first breeds until its "maximum longevity" (specified by user) is reached; animals can breed until they die.

Demography: Survival probabilities are density dependent when the population is below carrying capacity. When the population exceeds carrying capacity, all age- and sex-classes are equally affected. Migration rates are also independent of age and sex. Life-history traits are modeled as discrete, sequential events (i.e. as seasonal events), rather than continuous through time.

Genetic Variation: As only one locus is tracked for changes in genetic variation, it is assumed that all loci in the genome are affected in an equal manner. Inbreeding effects are modeled in one of two ways: the Heterosis model or the Recessive Lethals model. However, in reality, both models affect most populations. Inbreeding only affects juvenile survival; increased disease susceptibility or decreased ability to adapt is not modeled. VORTEX assumes that all founding genetic diversity is unique.

Other: A catastrophe only impacts the population during the year that it occurs.

Other attributes:

Variation in reproduction and mortality should be determined for studies whose focus is the conservation of a population, as high variance will influence the population stability. Variance in reproduction and mortality has two components: demographic stochasticity and variation from environmental stochasticity and catastrophes. Demographic stochasticity is modeled by binomial sampling to represent each individual's sex, reproduction, litter size, migration and death. Mortality and reproduction are sex- and age-class specific (i.e. animals can be grouped into younger than reproductive age, reproductive age, and older than reproductive age) and users can specify a "maximum longevity" age for the animals. Environmental stochasticity is also modeled by binomial sampling for each sex and age class. All individuals in a population are affected simultaneously by environmental effects, but the impact on each individual is determined from the variance (which is specified by the user - the higher the variance, the greater the impact on some individuals).

The effect of environmental variation on carrying capacity is modeled from a random normal distribution. When the carrying capacity (user-specified) is exceeded, each age class is truncated according to a probability function, and each animal has an equal probability of being removed from the population. Reproduction, mortality, and carrying capacity for each sex-age class are completely correlated with environmental variation. Reduction in breeding potential as a result of density dependence can be modeled using a polynomial

function describing the relationship between population size and breeding probability. The program can model either a positive response to low-density scenarios, Allee effects (negative response of population to low-density), or even more complicated relationships.

One of the most significant attributes of VORTEX is that both monogamous and polygamous mating systems can be modeled. However, mating pairs are randomly recombined after each year, so the monogamous model cannot produce accurate results for species that are faithful for multiple years. It is important to note that some versions of VORTEX model monogamous breeding differently and therefore produce different results. Version 5 (and lower) will pair males with females regardless of the female's ability to breed. This will be useful for most scenarios since females are often the limiting sex. Higher versions of VORTEX will only pair males to mating partners. Therefore, higher versions are better for monogamous breeding systems or if breeding males are the limiting factor.

Catastrophes are modeled as independent events that only affect reproduction and survival during the year that they occur. Any number of catastrophes can be modeled with VORTEX. Users can specify each catastrophe's probability of occurrence and its impact on survivorship. Migration is independent of sex and age and is modeled as an annual probability that an individual will move between subpopulations. VORTEX can be used to track the dynamics of local extinctions and recolonizations.

Changes in genetic variation can be modeled by VORTEX. Each individual is randomly assigned two alleles, and the alleles are transmitted according to the Infinite Alleles Model. Users specify the severity of inbreeding depression, and inbred individuals have a lower rate of juvenile survival. Inbreeding is modeled in two different ways: 1) the Heterosis Model or 2) the Recessive Lethals Model. In the Heterosis model, homozygotes have reduced fitness compared with heterozygotes. The user defines the number of "lethal equivalents" or severity of selection against homozygotes. In the Recessive Lethals model, selection occurs against individuals that are homozygous for the recessive (lethal) allele, and therefore, the highly deleterious alleles are removed from the population. All individuals begin with a recessive lethal allele and a dominant non-lethal allele. Since only one locus is modeled, the death rate is slightly higher than would be expected for a natural population, because recessive lethals are all at the same locus rather than spread throughout the genome. Therefore, this model overestimates the impact of inbreeding in many wildlife populations.

ALEX (Possingham *et al.* 1992)

ALEX was developed to examine the impact of environmental variation (e.g. timber harvesting) on species survival, particularly for organisms that experience much habitat variation among patches. The program can estimate extinction probabilities of the metapopulation, the time to extinction, and the time during which each habitat patch is unoccupied (local extirpation). It is especially

useful for species that use different habitat types or prefer certain stages of vegetation succession.

Strengths:

ALEX is particularly useful because of its flexibility in modeling catastrophes. A number of different catastrophes can be represented, but particularly those that influence or depend on habitat quality. As such, the program is excellent for modeling preferential movements of animals to better quality habitat areas. This program can examine up to 45 populations, with each population containing about 32 000 individuals. It is therefore not restricted to species with low fecundity. As with VORTEX, default values are provided for most parameters, so lack of knowledge about some population factors will have less of an impact on results.

ALEX is also capable of performing sensitivity analyses. This is a procedure used to determine how the parameters of a population are affected by the outcome of a particular course of action (i.e. it determines the sensitivity of the outcome on its parameters).

Limitations:

When using ALEX to model population viability, only the fate of the limiting sex is followed. In most cases, this is the female. Consequently, differences in mating systems or social structure cannot be examined. Furthermore, all populations within a metapopulation are modeled the same way.

Not all correlations between factors affecting population viability can be examined with this program. Correlation between patches can be modeled, but only between the original "reference" patch and the other patches. Correlation between environmental variation and reproduction can also be modeled, but not the correlation between environmental variation and mortality. ALEX cannot model the impacts of changes in genetic variation. Lindenmayer *et al.* (1995) found that the program requires much prior knowledge to operate and although default values are provided for most parameters, the package is designed for use with the aid of the architect.

Assumptions:

All populations within a metapopulation have the same demographic parameters. Populations are at a stable age distribution at the beginning of the simulation. Only adults are capable of breeding, but all adults can breed until they die.

Other attributes:

ALEX incorporates three life-history stages into its population model: newborn (<1yr), juveniles (0-5 yrs), and adults (>5 yrs). Birth and death rates are modeled from a random binomial distribution. A density-dependence model is incorporated when a population exceeds carrying capacity. When this occurs, the youngest individuals are removed from the population until the population size fits the carrying capacity again.

Up to three types of catastrophes can be modeled. Catastrophes only directly affect the population size in the year that they occur, but affect habitat and breeding success in subsequent years. To model the frequency of each catastrophe, the user can specify an annual probability of the occurrence of each catastrophe. The annual probability will either be specified as habitat-dependent (e.g. fire) or density-dependent (e.g. disease). Catastrophes can either occur independently within patches, or simultaneously.

ALEX is particularly useful for monitoring movements of animals between patches. ALEX models two types of movements between patches, each with a different occurrence probability that varies between age classes and density in the source patch. To model "migration", each individual has a probability of migrating once the density in a patch exceeds the carrying capacity by a specified proportion. The user specifies the average distance that an individual can move, while the direction of movement is randomly determined. Thus, the probability of death increases with increasing distance between patches. Individuals that emigrate in a direction away from another patch will die. Also, if only one patch is examined, all emigrating animals will be lost from the population. "Diffusion", on the other hand, models movements between adjacent or connected patches. This model only works when more than one subpopulation is examined. The number of animals that can diffuse is defined by the common length of the boundary - the greater the common region, the greater the number of animals that can move between patches. Since movement is simply between

connected patches, there is no mortality associated with diffusion. The user can specify preferential movements of animals to better habitat.

RAMAS (Risk Analysis and Management Alternatives Software)

There are several RAMAS programs, each with a separate objective for population viability analysis.

RAMAS/Age (Ferson and Akçakaya 1990)

RAMAS/Age was developed to analyze age-structured population dynamics. It models a single population's growth and development as it is affected by various factors. Age predicts the number of individuals in each age class in future years and estimates the probability of population growth or extinction.

Strengths:

RAMAS/Age is particularly useful for species with high fecundity, as it was developed to examine very large population sizes. It is designed so that the user can obtain conservative risk estimates by using the default parameters when information about the population is incomplete.

Limitations:

RAMAS/Age is a very limited program. It would be useful to answer specific questions about the interactions between various factors and age

distribution in a population. However, much information is required before the program can be used and the results cannot be extended to other populations (Ferson and Akçakaya 1991). RAMAS/Age examines neither catastrophic events, nor the effects of systemic pressures on population viability. The program also does not incorporate inbreeding depression or metapopulation structure into the models.

Assumptions:

RAMAS/Age only models populations with a polygamous breeding system. As the sex ratio is incorporated in the fecundity estimate, the program does not model stochasticity in mate availability.

Other attributes:

RAMAS/Age is capable of performing sensitivity analyses to identify the important demographic factors in endangered species populations. The user specifies survival and fecundity rates, as well as characteristics about density-dependence models, sex ratios and migration to determine how different age classes of a specific population are influenced by these factors.

RAMAS/Stage (Ferson 1990)

RAMAS/Stage was developed for the population viability analysis of species whose demographic characteristics are structured by life stages, rather

than by age. The program is used to analyze discrete-time models for a population. It is especially useful for species with complex life-history traits.

Strengths:

RAMAS/Stage differs from RAMAS/Age in that the program is able to incorporate both systemic pressures and catastrophic events into the population viability model. Because RAMAS/Stage does not assume a polygamous mating system, stochastic variation in sex ratio and availability of mates can also be modeled. This can be useful for monogamous populations in which the male can sometimes be the limiting sex. RAMAS/Stage has a number of templates that are easy to customize and can be used to model the population viability of many taxa (such as mammals, insects, fish, birds and plants). Although Stage can be used to construct simple models based on the developmental stages of a population, its primary strength lies in its ability to construct more complex life models that can incorporate multidimensional functions.

Limitations:

As with RAMAS/Age, RAMAS/Stage cannot incorporate inbreeding depression or metapopulation structure into the models. Also, the program is primarily useful for answering a specific question about a specific population (Ferson 1991). The results should not be extended to other populations.

Other attributes:

RAMAS/Stage examines how environmental stochasticity affects populations through time and can perform risk assessments for any function within a life stage. RAMAS/Stage was derived from RAMAS/Age to incorporate more complex models that are based on stage-specific, rather than age-specific, survival rates. This is important for representing certain (rare) phenomena that can cause individuals to skip stages, revert to previous stages, or produce offspring of different status. Consequently, Stage is able to model the complex life histories of certain species.

RAMAS/Metapop (Akçakaya 1996)

RAMAS/Metapop was designed to assess the impact of humans on fragmented populations and to explore management options such as translocations, reintroductions, and reserve design. The software was developed from RAMAS/Space to examine stage-structured metapopulation dynamics. This program can also be useful for single populations.

Strengths:

RAMAS/Metapop can model the effects of spatial structure on population viability. It can also be used to analyze age- or stage-structured populations. The software accommodates multiple populations with very large sizes and therefore incorporates a density-dependent function. Up to 160 populations can be analyzed with 20 stages and 100 different types of age or stage matrices each.

Limitations:

RAMAS/Metapop cannot model the effects of inbreeding depression on population viability.

Assumptions:

Metapop assumes polygamous breeding and that the sex ratio is incorporated in the fecundity estimate. Therefore, it does not model stochasticity in mate availability.

Other attributes:

The primary difference between RAMAS/Metapop and other RAMAS programs is that Metapop can incorporate metapopulation structure into the population projections. Many species occur as metapopulations in nature and the spatial structure of the different habitats used has an important influence on the population dynamics. Metapop can incorporate variables such as dispersal and recolonization, as well as the configuration of the populations and similarities of environmental patterns experienced by them. Each of these factors can affect metapopulation dynamics.

RAMAS/Metapop differs from RAMAS/Age in that it incorporates both catastrophic events and systemic pressures into the population viability models. Users can specify the probability of occurrence of catastrophic events as well as their impact on survivorship. This program can also incorporate a density-

dependent ceiling model to reflect competition based on breeding territories. When the population size exceeds the carrying capacity, the population is reduced to its carrying capacity by randomly eliminating individuals.

Metapop can estimate the risk of metapopulation decline and species extinction. As well, it can estimate time to extinction and provide probabilities of population growth. The program outlines estimates of the abundance of each population and the metapopulation through time.

RAMAS/Space (Akçakaya and Ferson 1992)

RAMAS/Space was developed to examine various strategies for reintroduction and translocation of some species.

Strengths:

This software package is particularly useful for large populations with high levels of fecundity. The program allows users to analyze multiple population models; it can examine 160 populations at a time that contain up to 2 billion individuals. Consequently, RAMAS/Space also incorporates a wide range of density-dependent processes. Lindenmayer *et al.* (1995) found that RAMAS/Space was the most user-friendly of the three packages they examined, as they felt that the package could be used with a limited understanding of population dynamics.

Limitations:

RAMAS/Space is not age or stage structured. These factors can be incorporated by also running either RAMAS/Age or RAMAS/Stage. RAMAS/Space also cannot accommodate different mating systems or social structure in different subpopulations. In most instances, this will not be important, as most subpopulations will have similar mating and social systems. RAMAS/Space is not suitable for small (i.e. captive) populations or where catastrophes are important, as it does not incorporate catastrophes into the model. If there are many unknown parameters for the population, this program is not as useful due to the number of assumptions that must be made. There are fewer default values provided than in VORTEX and ALEX, and it is not intended to be run with the assistance of the architect. Contrary to the opinion of Lindenmayer *et al.* (1995), Akçakaya and Ferson (1990) found that Space was difficult to use without a strong understanding of population viability.

Assumptions:

Catastrophes are represented by the extreme left tails of the distribution of population growth rates.

Other attributes:

In RAMAS/Space, demographic variability is modeled by generating random numbers from a binomial distribution for survivorship and from a Poisson distribution for number of offspring. Different subpopulations can have different demographic parameters (e.g. growth rate, carrying capacity, survivorship). The

correlation between subpopulations can also be examined. The user either specifies this level of correlation or it can be generated from a function that relates level of similarity between patches (based on growth rates) with distance between them. Environmental stochasticity is modeled by randomly specifying annual growth rate from a log normal distribution. Carrying capacities are not affected by environmental variation.

RAMAS/Space can incorporate four density-dependence models (logistic growth, Malthusian growth (density-independent, exponential growth when the population is unchecked by environmental or social constraints), the Ricker function (modified exponential growth) and Allee effects (undercrowding)). The density-dependent function can also be specified by the user to incorporate both overcrowding and undercrowding simultaneously - in which the population is at a maximum at an intermediate density and growth rate decreases as the population either increases or declines. Each subpopulation can have its own density-dependent function.

Migration is modeled by specifying probabilities of movements between each pair of subpopulations. This is often determined from the physical distance between subpopulations. RAMAS/Space can also incorporate corridors or barriers between subpopulations. Migration rates can also be density-dependent, as specified by the user. Direction of migration can also be specified to reflect asymmetry in the permeability between two patches (i.e. it allows for the possibility that it is easier to move from A to B than to move from B to A).

GAPPS (Harris *et al.* 1986)

GAPPS was developed specifically to examine grizzly bear populations. Since that time it has frequently been used to model other large mammalian populations (Dixon *et al.* 1991, Dobson *et al.* 1992).

Strengths:

GAPPS is more flexible than VORTEX in modeling the effects of inbreeding depression on population viability. Also, the package can model different mating systems.

Limitations:

GAPPS cannot incorporate metapopulation structure into its models. Also, systemic pressures are not included in the population projections. Consequently, environmental impacts such as habitat decline cannot be examined, although they may have a very significant influence on the viability of the population.

Other attributes:

The effects of changes in the levels of genetic variation in the population can be simulated using GAPPS. The modeling of inbreeding depression in populations is more flexible with this program than with VORTEX, which only models the effect of inbreeding on juvenile survival. Both polygamous and monogamous breeding systems can be modeled. The monogamous breeding model gives lower projections than the polygamous model (Brook *et al.* 1999). In

order to model the effect of catastrophic events on population viability, the user can specify the probability of a particular catastrophe occurring and the impact the catastrophe has on survivorship. Stochastic variation in sex ratio and the availability of mates can also be modeled, which can be very important for monogamous populations (see VORTEX).

INMAT (Mills and Smouse 1994)

INMAT was developed to examine the short-term effects of inbreeding under stochasticity.

Limitations:

INMAT cannot incorporate metapopulation structure into its models. The program also does not model systemic pressures, and consequently, environmental effects such as habitat decline cannot be examined. INMAT does not incorporate any catastrophic events into its model of population viability. Managers should therefore be cautious when using the population projections derived from INMAT since they will be higher than will be possible to achieve in a natural population where catastrophes may occur.

Assumptions:

INMAT assumes polygamous breeding in all populations examined. The sex ratio is incorporated in fecundity estimates. Consequently, INMAT cannot model stochasticity in mate availability.

Other attributes:

INMAT uses a more flexible model than VORTEX for incorporating the effects of inbreeding depression on population viability. The program does not, however, allow the user to specify the initial relatedness within the population. INMAT can model competition based on breeding territories by incorporating a density-dependent "ceiling model". When the population exceeds its carrying capacity, it is reduced randomly to the appropriate size.

Table 4. Population Viability Analysis (PVA) computer packages

Package	Objective	Strengths	Limitations	References
VORTEX	Developed to determine how species survival is affected by the interaction between genetic factors and population demography.	- Useful for organisms with low fecundity and long life spans. - Can model the effects of genetic changes in a population. - Can track the fate of individuals, rather than just cohorts. - Allows the user to supplement or harvest the population. - Can model both monogamous and polygamous mating systems.	- A maximum of only 20 populations can be examined, with maximum population sizes of 1000. - Heavy data requirements. - Not useful for populations strongly affected by predator-prey interactions. - Inbreeding effects are modeled in one of two ways, neither of which is realistic.	Lacy and Kreeger 1992, Lacy 1993, Miller and Lacy 1995
ALEX	Developed to examine the impact of environmental variation on species survival, particularly for organisms that experience much habitat variation among patches.	- Flexibility in modeling catastrophes - Useful for modeling preferential movements of animals to better quality habitat areas. - Can examine up to 45 populations, with population sizes of about 32 000. - Useful for species that use different habitat types or prefer certain stages of vegetation succession. - *Can perform sensitivity analyses* - Incorporates 3 life-history stages into its population model.	- Cannot model the impacts of changes in genetic variation. - Only the fate of the limiting sex is followed. - Cannot model differences in mating systems or social structure. - All populations within a metapopulation are modeled the same way. - Requires much prior knowledge to operate.	Possingham *et al.* 1992
RAMAS/ Age	Developed to analyze age-structured population dynamics.	- Useful for species with high fecundity. - Designed so that the user can obtain conservative risk estimates by using the default parameters when information about the population is incomplete.	- Requires much information and the results cannot be extended to other populations. - Can only model populations with a polygamous breeding system. - Cannot model effects of inbreeding depression or metapopulation structure. - Cannot examine catastrophic events or the effects of systemic pressures on population viability.	Ferson and Akçakaya 1990

Table 4. Population Viability Analysis (PVA) computer packages (continued)

Package	Objective	Strengths	Limitations	References
RAMAS/ Stage	Developed for the PVA analysis of species whose demographic characteristics are structured by life stages, rather than by age.	- Useful for species with complex life-history traits. - Can model both systemic pressures and catastrophic events. - Can model both monogamous and polygamous mating systems. - Can model environmental stochasticity. - Can perform risk assessments for any function within a life stage.	- Cannot incorporate inbreeding depression or metapopulation structure into the models. - The results cannot be extended to other populations.	Ferson 1990
RAMAS/ Metapop	Developed to assess the impact of humans on fragmented populations and to explore management options such as translocations, reintroductions, and reserve design.	- Can examine stage-structured metapopulation dynamics. - Can model the effects of spatial structure on population viability. - Up to 160 populations can be analyzed with 20 stages and 100 different types of age or stage matrices each. - Incorporates both catastrophic events and systemic pressures.	- Cannot model the effects of inbreeding depression on population viability. - Can only model polygamous breeding systems. - Does not model stochasticity in mate availability.	Akçakaya 1996
RAMAS/ Space	Developed to examine various strategies for reintroduction and translocation of some species.	- Can examine 160 populations at a time that contain up to 2 billion individuals. - Incorporates a wide range of density-dependent processes.	- RAMAS/Space is not age or stage structured - Different subpopulations are modelled the same way. - Cannot model catastrophes. - Not useful if there are many unknown parameters for the population.	Akçakaya and Ferson 1992
GAPPS	Developed specifically to examine grizzly bear populations, but has recently been used to model other large mammalian populations.	- More flexible than VORTEX in modeling the effects of inbreeding depression - Both polygamous and monogamous breeding systems can be modelled.	- Cannot incorporate metapopulation structure into its models. - Cannot incorporate systemic pressures into population projections.	Harris *et al.* 1986)
INMAT	Developed to examine the short-term effects of inbreeding under stochasticity.	- Designed to model low growth rate "ungulates", medium growth rate "felids" and high growth rate "rodents". - Examines inbreeding effects on survival and fertility depression. - Incorporates habitat fragmentation and isolation into model.	- Cannot incorporate metapopulation structure into its models - Does not model systemic pressures. - Cannot incorporate catastrophic events.	Mills and Smouse 1994

5.3) Conclusions

Due to the vast differences between models, it is important that the user explicitly state the question being asked to ensure that the correct program is used. If more than one program is applicable, it is recommended that they all be tried and the results of each program be compared before management decisions are made. This will ensure that only the effects of the important factors are recognized, while those that result in significant differences due to the model choice are ignored. Brook *et al.* (1999) recommend that risk estimates be conservative and all possible threats to the population be included in the analyses. If this is true, then VORTEX should be the best package as it incorporates the most factors. Unfortunately, as more threats are included, the model becomes more complex and more assumptions are made. Consequently, the population projections may no longer be accurately predicted.

Brook *et al.* (1997) found that INMAT, GAPPS, RAMAS/Age, RAMAS/Metapop and VORTEX all gave similar, but highly unrealistic results under density-independent conditions. When density-dependent factors were included, the population projections were still too high and no longer consistent among programs.

6. Recommendations

As outlined in Section 3.2, there are a number of simple procedures that, when applied to the Hook Lake Wood Bison Recovery Project, will act to minimize the loss of diversity and adaptation to the captive environment in this

population. If the effective size of this population is maximized, then loss of diversity will be minimized. N_e is greatest in a population when there are no fluctuations in population size, minimal differences in reproductive success between individuals, and equal sex ratios. To reduce population size fluctuations, the HLWBRP population should be managed at or near the carrying capacity of its holding region. If the population does decrease in size due to a management decision or natural event, it should be allowed to increase again as quickly as possible to avoid loss of diversity during the bottleneck. Ensuring that all animals are given a chance to reproduce can minimize differences in reproductive success. In order for each individual's reproductive success to be known, parentage should be established through the use of DNA microsatellite analysis. Maintaining an equal sex ratio is more difficult. In bison, a polygynous species, only a few males are allowed the opportunity to reproduce each year. As a result, establishing an equal sex ratio within the population will only increase the number of unsuccessful males if intense management for equal reproductive success is not performed on the population.

Adaptation to the captive environment can be minimized by continued gene flow with the wild population, equalization of family size (discussed above), increased generation length, and increased similarity between the captive and natural environment. Continued gene flow with the wild population is not feasible for the HLWBRP, as any introduction of animals would endanger the attempts to obtain disease-free status for this population. As the processes of genetic drift and selection only occur between generations, lengthening the generation time

would decrease the loss of diversity loss and adaptation per year. However, this should only be done when the population has reached carrying capacity. An increased generation length would have the added advantage of slowing the rate of growth in the population, resulting in fewer surplus animals. To ensure that animals would have an opportunity to reproduce before they die, one possibility would be to breed animals when they have reached reproductive age and then once more when they are nearing the end of their reproductive life. If space is an issue, the first-born animal could then be declared surplus. The HLWBRP is likely as similar to natural conditions as it can be, while still allowing for animal handling and ensuring the health of the individuals.

While all of the above strategies will act to minimize the loss of genetic diversity through time, they do not allow for an estimate of the loss of this diversity. Also, they are unable to make recommendations as to which animals should be bred together to maximize the diversity in the population. Establishing reproductive strategies to maximize diversity will be further assisted by the use of a program such as GENES pedigree analysis or PM2000 software to determine the relationship between individuals in the population (Section 4). With this information and a measure of the genetic importance of each individual, animals with high genetic importance can be selected as potential breeders. We recommend kinship value (KV) as the preferred measure of individual genetic importance. This measure is based on mean kinship (MK), which has proven to be one of the most effective measures at retaining the diversity within a population (Section 4.2). Kinship value is weighted by the age structure of the

population, thus ensuring that individuals who may soon be unavailable as breeders because of their age, but are important genetically, are given an opportunity to breed.

A more global estimate of the likelihood of population persistence can be obtained by performing population viability analysis (PVA, Section 5). These analyses can combine the genetic information derived above with estimates of demographic and environmental stochasticity to model the likelihood of long-term population persistence. With the use of PVA software packages, we can determine the potential outcome of certain management practices and can thereby identify the optimal strategy for a particular herd.

Each software program was created to answer a specific question. Consequently, the packages differ with respect to the information they require, as well as their strengths, limitations, and assumptions. Certain demographic factors will be deemed more important for one program and less important for another program. As a result, the population projections of each package often differ significantly. As indicated in Section 5.2, the selection of PVA package should be based on the objective of the study, and how well the program's requirements match the available population data. For any population modeling study, we recommend the use of at least two PVA software packages in order to avoid biases introduced by a particular package. Results that differ significantly based on the model choice are considered questionable and should be ignored.

For the Hook Lake Wood Bison Recovery Project, we recommend the use of two PVA programs: VORTEX and INMAT. Both of these packages can model

genetic and demographic stochasticity in populations. As well, they can estimate the probability of extinction and the time to extinction. VORTEX is particularly useful for tracking individuals in populations of large mammals with low fecundity and long life spans. This program was specifically developed to establish conservation strategies for endangered species. VORTEX may be the best package for this study as it incorporates the most factors for its analysis. Its projection of population viability is likely conservative, as it includes all factors that influence the viability of the animals. Although VORTEX is probably the most detailed PVA package currently available, the large amount of information required can create problems if this information is not available for the population in question. However, for the HLWBRP, most of this information is either directly available, or can be estimated from other captive wood bison populations like Elk Island National Park.

Inbreeding effects can be better incorporated by INMAT since VORTEX has only two options for modeling inbreeding, neither of which is realistic. VORTEX may provide a low estimate of the rate of loss of genetic diversity since it assumes an equal probability of mating. However, because INMAT does not allow the user to specify initial relatedness within a population, it is impossible to include information about genetic importance, previously derived using MK and GENES.

Although both VORTEX and INMAT have limitations, we believe that these limitations are of little concern for the Hook Lake Wood Bison Recovery Project. For instance, we do not expect catastrophes or systemic environmental

pressures to influence the viability of this population. Because we are dealing with a managed captive population, variation in mate availability should be less of an issue than in wild populations.

ACKNOWLEDGEMENTS

The Hook Lake Wood Bison Recovery Project is a cooperative conservation effort between the Deninu Kue' First Nation Aboriginal Wildlife Harvesters' Committee and the Government of the Northwest Territories. Operations and management of the HLWBRP is a collaborative effort involving many people. The AWHC board members (R. Sayine Sr., R. Ekinla, W. Delorme, D. Giroux, R. King, F. Lafferty), and project staff (S. Cuthbert and K. Delorme) in Fort Resolution have played a key role in the continuing and evolving success of the project. R. Boucher, T. Unka, D. Balsillie, C. Gates, D. Beaulieu, P. King, R. Sayine Jr. & J. Unka are recognized for their fundamental contributions to the project, as well as the untold number of student volunteers contributing their time and enthusiasm to the HLWBRP over the years. RWED staff, T. Ellsworth and B. Elkin, are especially acknowledged for their support and unwavering dedication and commitment to this long-term project.

This report was funded by the Department of Resources, Wildlife & Economic Development through a Service Contract (SC 361545) with the senior author.

We thank M. Sutherland for meticulous editing of previous versions of the manuscript.

LITERATURE CITED

Akçakaya, H.R. 1996. *RAMAS® Metapop: viability analysis for stage-structured metapopulations, Version 2.0.* Applied Biomathematics, Setauket, NY.

Akçakaya, H.R. and S. Ferson. 1990. *RAMAS / SPACE.* Applied Biomathematics, Setauket, NY.

Akçakaya, H.R. and S. Ferson. 1992. *RAMAS/Space: spatially structured population models for conservation biology, Version 1.3.* Applied Biomathematics, Setauket, NY.

Allard, R.W. 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. Journal of Heredity 79:225-238.

Allee, W.C., A.E. Emerson, O. Park, T. Park, and K.P. Schmidt. 1949. Principles of animal ecology. W.B. Saunders, Philadelphia, PA. 837 pp.

Allendorf, F.W. and R.F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. Pages 57-86 *in* Soulé, M.E., ed. Conservation biology: the science of scarcity and diversity. Sinauer Associates Inc., Sunderland, MA.

Allendorf, F.W. and N. Ryman. 2002. The role of genetics in population viability analysis. Pages 50-85 *in* Beissinger, S.R., and McCullough, D.R, eds. Population viability analysis. University of Chicago Press, Chicago, IL.

Asquith, N.M. 2001. Misdirections in conservation biology. Conservation Biology 15:345-352.

Ballou, J.D. and T.J. Foose. 1996 Demographic and genetic management of captive populations. Pages 263-282 *in* Kleiman, D.G., ed. Wild mammals in captivity: principles and techniques. University of Chicago Press, Chicago, IL.

Ballou, J.D. and R.C. Lacy. 1995. Identifying genetically important individuals for management of genetic diversity in pedigreed populations. Pages 76-111 *in* Ballou, J.D., Gilpin, M., Foose, T.J., eds. Population management for survival and recovery. Columbia Press, New York, NY.

Barrett, S.C.H. and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature. 352:522-524.

Beissinger, S.R. 2002. Population viability analysis: past, present, future. Pages 5-17 *in* Beissinger, S.R. and D.R. McCullough. 2002. Population viability analysis. University of Chicago Press, Chicago, IL.

Beissinger, S.R. and D.R. McCullough. 2002. Population viability analysis. University of Chicago Press, Chicago, IL. 577 pp.

Berger, J. 1990. Persistence of different-sized populations: an empirical assessment of rapid extinctions in bighorn sheep. Conservation Biology 4:91-98.

Berger, J., and C. Cunningham. 1994. Bison: mating and conservation in small populations. Columbia University Press, New York, NY. 330 pp.

Bijlsma, R., J. Bundgaard, and A.C. Boerema. 2000. Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. Journal of Evolutionary Biology 13:502-514.

Bijlsma, R., J. Bundgaard, and W.F. Van Putten. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. Journal of Evolutionary Biology 12:1125-1137.

Boyce, M. 1992. Population viability analysis. Annual Review of Ecology and Systematics 23:481-506.

Briscoe, D.A., J.M. Malpica, A. Robertson, G.J. Smith, R. Frankham, R.G. Banks, and J.S.F. Barker. 1992. Rapid loss of genetic variation in large captive populations of *Drosophila* flies: implications for the genetic management of captive populations. Conservation Biology 6: 416-425.

Brook, B.W., L. Lim, R. Harden, and R. Frankham. 1997. Does population viability analysis software predict the behaviour of real populations? A retrospective study on the Lord Howe Island woodhen *Tricholimnas sylvestris* (Sclater). Biological Conservation 82:119-128.

Brook, B.W., J.R. Cannon, R.C. Lacy, C. Mirande and R. Frankham. 1999. Comparison of the population viability analysis packages GAPPS, INMAT, RAMAS and VORTEX for the whooping crane (*Grus americana*). Animal Conservation 2:23-31.

Brook, B. W., J. J. O'Grady, A. P. Chapman, M. A. Burgman, R. Akcakaya, and R. Frankham. 2000. Predictive accuracy of population viability analysis in conservation biology. Nature. 404: 385–387.

Bryant, E.H. and D.H. Reed. 1999. Fitness decline under relaxed selection in captive populations. Conservation Biology 13:665-669.

Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology. 63: 215-244.

Caughley, G, and A, Gunn. 1996. Conservation biology in theory and practice. Blackwell Science Inc, Cambridge, MA. 459 pp.

Charlesworth, D. and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Connelly, R., W. Fuller, G. Wobeser, R. Mercredi, and B. Hubert. 1990. Northern diseased bison report of the Environmental Assessment Panel. Environmental Assessment Review Office, Report 35. Hull, Quebec. 47 pp.

Coulson, T., G. M. Mace, E. Hudson, and H. Possingham. 2001. The use and abuse of population viability analysis. Trends in Ecology and Evolution. 16: 219–221.

Crow, J.F. and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row, New York, NY. 591 pp.

Darwin, C.R. 1868. The variation of animals and plants under domestication. John Murray, London, UK. 2 volumes. 494 & 568 pp.

Dixon, A.M., Mace, G.M., Newby, J.E. and Olney, P.J.S. 1991. Planning for the re-introduction of scimitar-horned oryx (Oryx dammah) and addax (Addax nasomaculatus) into Niger. Symposium of the Zoological Society of London 62: 201-216.

Dobson, A.P., Mace, G.M., Poole, J. and Brett, R.A. 1992. Conservation biology: the ecology and genetics of endangered species. In Genes in ecology: 405-430. Berry, R. J., Crawford, T.J. and Hewitt, G.M. (Eds). Oxford: Blackwell Scientific

Ellegren, H., G. Hartman, M. Johansson, and L. Andersson. 1993. Major histocompatibility complex monomorphism and low levels of DNA fingerprinting variability in a reintroduced and rapidly expanding population of beavers. Proceedings of the National Academy of Sciences USA 90:8150-8153.

Ferson, S. 1990. *RAMAS/Stage: generalised stage-based modeling for population dynamics*. Applied Biomathematics, Setauket, NY.

Ferson, S. and H.R. Akçakaya. 1990. *RAMAS® Age users manual: modeling fluctuations in age-structured populations.* Applied Biomathematics, Setauket, NY.

Ferson, S. and H.R. Akçakaya. 1991. *RAMAS / AGE.* Applied Biomathematics, Setaukety, NY.

Ferson. S. 1991. *RAMAS / STAGE.* Applied Biomathematics, Setauket, NY.

Fitzsimmons, N.N., S.W. Buskirk, and M.H. Smith. 1995. Population history, genetic variability, and horn growth in bighorn sheep. Conservation Biology 9:314-323.

Frankel, O.H. and M.E. Soulé. 1981. Conservation and evolution. Cambridge University Press, Cambridge, UK. 327 pp.

Frankham, R. and D.A. Loebel. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: rapid genetic adaptation to captivity. Zoo Biology 11:333-342.

Frankham, R., J.D. Ballou, and D.A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, New York, NY. 671 pp.

Franklin, I.R. 1980. Evolutionary change in small populations. Pages 135-149 *in* Soulé, M.E. and Wilcox, B., eds. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates Inc., Sunderland, MA.

Fuller, W.A. 2002. Canada and the "buffalo", Bison bison: a tale of two herds. Canadian Field Naturalist 116: 141-159.

Gaona, P., P. Ferraras, and M. Delibes. 1998. Dynamics and viability of a metapopulation of the endangered Iberian lynx (*Lynx pardinus*). Ecological Monographs 68:349-370.

Gates, C.C., B.T. Elkin, and D.C. Beaulieu. 1998. Initial results of an attempt to eradicate bovine tuberculosis and brucellosis from a wood bison herd in northern Canada. In: International Symposium on Bison Ecology and Management in North America. L. Irby and J. Knight, Eds: 221-228. Montana State University, Bozeman, MO.

Gates, C.C., R.O. Stephenson, H.W. Reynolds, C.G. van Zyll de Jong, H. Schwantje, M. Hoefs, J. Nishi, N. Cool, J. Chisholm, A. James, and B. Koonz. 2001. National Recovery Plan for the Wood Bison *(Bison bison athabascae).* National Recovery Plan No. 21. Recovery of Nationally Endangered Wildlife (RENEW). Ottawa, Ontario. 50 pp.

Geist, V. 1991. Phantom subspecies: the wood bison, *Bison bison "athabascae"* Rhoads 1897, is not a valid taxon, but an ecotype. Arctic 44:283-300.

Gilligan, D.M., L.M. Woodworth, M.A. Montgomery, D.A. Briscoe, and R. Frankham. 1997. Is mutation accumulation a threat to the survival of endangered populations? Conservation Biology 11:1235-1241.

Gilpin, M.E. and M.E. Soulé. 1986. Minimum viable populations: processes of species exitnction. Pages 19-34 *in* Soulé, M.E., ed. Conservation biology: the science of scarcity and diversity. Sinauer Associates Inc, Sunderland, MA.

Goodman, D. 1987. The demography of chance extinction. Pages 11-34 *in* Soulé, M.E., ed. Viable populations for conservation. Cambridge University Press, Cambridge, UK.

Guthrie, R.D. 1990. Frozen fauna of the Mammoth Steppe: the story of Blue Babe. University of Chicago Press, Chicago, IL. 323 pp.

Haig, S.M., J.D. Ballou, and N.J. Casna. 1994. Identification of kin structure among Guam rail founders: a comparison of pedigrees and DNA profiles. Molecular Ecology 3:108-119.

Harris, R.B., L.H. Metzgar, and C.D. Bevin. 1986. *GAPPS: generalized animal population projection system. Version 3.1.* Montana Co-operative Wildlife Research Unit, University of Montana, Missoula, MT.

Hedrick, P.W. 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. Heredity 73:363-372.

Hedrick, P.W. and S.T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecological Systematics 31:139-162.

Hewitt,C.G. 1921. The conservation of wildlife in Canada. C. Scribner's Sons, New York, NY. 334 pp.

Hoelzel, A.R., J. Halley, S.J. O'Brien, C. Campagna, T. Arnbom, B. Le Boeuf, K. Ralls, and G.A. Dover. 1993. Elephant seal genetic variation and the use of simulated models to investigate historical population bottlenecks. Journal of Heredity 84:443-449.

James, J.W. 1971. The founder effect and response to artificial selection. Genetical Research 12:249-266.

Johnsson, J.L. and M.V. Abrahams. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout

(*Oncorhyncus mykiss*): an experimental study. Canadian Journal of Fisheries and Aquatic Sciences 48:243-247.

Joly, D.O. and F. Messier. 2001. Limiting effects of bovine brucellosis and tuberculosis on wood bison within Wood Buffalo National Park. Final report submitted to Wood Buffalo National Park, Heritage Canada, Fort Smith, NT. 127 pp.

Kelly, M.J. 2001. Lineage loss in Serengeti cheetahs: consequences of high reproductive variance and heritability of fitness on effective population size. Conservation Biology 15:137-147.

Kimura, M. and J.F. Crow. 1963. On the maximum avoidance of inbreeding. Genetical Research 4:399-415.

Kimura. M. 1983. The neutral theory of molecular evolution. Cambridge University Press, Cambridge, UK. 367 pp.

Lacy, R.C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1:143-158.

Lacy, R.C. 1990. *GENES: pedigree analysis software*. Chicago Zoological Park, Brookfield, IL.

Lacy, R.C. 1993. VORTEX: a computer simulation for use in population viability analysis. Wildlife Research 20:45-65

Lacy, R.C. 1994. Managing genetic diversity in captive populations of animals. Pages 63-89 *in* Bowles, M.L. and Whelan, C.J., eds. Restoration of endangered species. Cambridge University Press, Cambridge, UK.

Lacy, R.C. 1995. Clarification of genetic terms and their use in the management of captive populations. Zoo Biology 14:565-578.

Lacy, R.C. 1997. Importance of genetic variation to the viability of mammalian populations. Journal of Mammalogy 78:320-335.

Lacy, R.C. 2000. Should we select genetic alleles in our conservation breeding programs? *Zoo* Biology 19:279-282.

Lacy, R.C. and T.J. Kreeger. 1992. *VORTEX: a stochastic simulation of the extinction process. Version 5.1*. Brookfield Zoo, Chicago Zoological Society, Chicago, IL.

Lacy, R.C., M. Borbat, and J.P. Pollak. 2003. VORTEX: A Stochastic Simulation of the Extinction Process.Version 9. Brookfield, IL: Chicago Zoological Society. [Online] URL: http://www.vortex9.org/vortex.html

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutation. Evolution 48:1460-1469.

Lande, R. and G. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pages 87-123 *in* Soulé, M., ed. Viable populations for conservation. Cambridge University Press, Cambridge, UK.

Leigh, E.G. 1975. Population fluctuations, community stability and environmental variability. Pages 87-123 *in* Cody, M.L. and Diamond, J.M., eds. Ecology and evolution of communities. Harvard University Press, Cambridge, MA.

Lindenmayer, D.B., M.A. Burgman, H.R. Akçakaya, R.C. Lacy, and H.P. Possingham. 1995. A review of the generic computer programs ALEX, RAMAS/space and VORTEX for modelling the viability of wildlife metapopulations. Ecological Modelling 82:161-174

Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. Pages 471-501 *in* Avise, J.C. and J.L. Hamrlick, eds. Conservation genetics: case histories from nature. Chapman and Hall, New York, NY.

Lynch, M. and W. Gabriel. 1990. Mutation load and the survival of small populations. Evolution 44:1725-1737.

Lynch, M., J. Conery, and R. Burger. 1995. Mutation accumulation and the extinction of small populations. American Naturalist 146:489-518.

MacArthur, R.H. and E.O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, NJ.

Maguire, L.A., G.F. Wilhere, and Q. Dong. 1995. Population viability analysis for red-cockaded woodpeckers in the Georgia piedmont. Journal of Wildlife Management 59:533-542.

Miller, P.S. and R.C. Lacy. 1995. *VORTEX: a stochastic simulation of the extinction process. Version 8.* IUCN/SSC Conservation Breeding Specialist Group. Apple Valley, MN.

Mills, L.S. and P.E. Smouse. 1994. Demographic consequences of inbreeding in remnant populations. American Naturalist 144: 412-431.

Mills, L.S., Hayes, S.G., Baldwin, C., Wisdom, M.J., Citta, J., Mattson, D.J. and Murphy, K. 1996. Factors leading to different viability predictions for a grizzly bear data set. Conservation Biology. 10: 863-873.

Moehlman, P.D., G. Amato, and V. Runyoro. 1996. Genetic and demographic threats to the black rhinoceros population in the Ngorongoro crater. Conservation Biology 10:1107-1114.

Montgomery, M.E., J.D. Ballou, R.K. Nurthern, P.R. England, D.A. Briscoe, and R. Frankham. 1997. Minimizing kinship in captive breeding programs. Zoo Biology 16:377-389.

Nishi, J.S., B.T. Elkin, T.R. Ellsworth, D.W. Balsillie, G.A. Wilson, and J. van Kessel. 2001. An overview of the Hook Lake Wood Bison Recovery Project: where have we come from, where are we now, and where we would like to go? Pages 215-233 in Rutley, B.D., ed., Bison are Back – 2000. Proceedings of the Second International Bison Conference, Aug 2-4, 2000, Edmonton, AB.

Nishi, J.S., B.T. Elkin, and T.R. Ellsworth. 2002a. The Hook Lake Wood Bison Recovery Project: Can a disease-free captive wood bison herd be recovered from a wild population infected with bovine tuberculosis and brucellosis? Ann. NY Acad. Sci. 969: 229-235.

Nishi, J.S., C. Stephen, and B.T. Elkin. 2002b. Implications of agricultural and wildlife policy on management and eradication of bovine tuberculosis and brucellosis in free-ranging wood bison of northern Canada Ann. NY Acad. Sci. 969: 236-244.

Novakowski, N.S. 1978. Status report on wood bison in Canada Bison bison athabascae. COSEWIC report. 22 pp.

Nunney, L. and K.A. Campbell. 1993. Assessing minimum viable population size: demography meets population genetics. Trends in Ecology and Evolution 8:234-239.

O'Brien, S.J. 1994. A role for molecular genetics in biological conservation. Proceedings of the National Academy of Sciences 91:5748-5755.

O'Brien, S.J., M.E. Roelke, L. Marker, A. Newman, C.A. Winkler, D. Meltzer, L. Colly, J.F. Evermann, M. Bush, and D.E. Wildt. 1985. Genetic basis for species vulnerability in the cheetah. Science 227:1428-1434.

Ogilvie, S.C. 1979. The park buffalo: the history of the conservation of the North American Bison. National and Provincial Parks Association of Canada, Calgary-Banff Chapter. Reid, Crowther and Partners, Calgary, AB. 70 pp.

Packer, C., A.E. Pusey, H. Rowley, D.A. Gilbert, J. Martenson, and S.J. O'Brien. 1991. Case study of a population bottleneck: lions of the Ngorongo Crater. Conservation Biology 5:219-230.

Pollak, J. P., R. C. Lacy and J. D. Ballou. 2002. Population Management 2000, version 1.163. Chicago Zoological Society, Brookfield, IL. [Online] URL: http://www.vortex9.org/pm2000.html

Polziehn, R.O., R. Beech, J. Sheraton, and C. Strobeck. 1996. Genetic relationships among North American bison populations. Canadian Journal of Zoology 74:738-749.

Polziehn, R.O., C. Strobeck, J. Sheraton, and R. Beech. 1995. Bovine mtDNA discovered in North American bison populations. Conservation Biology 9:1638-1643.

Possingham, H.P., I. Davies, J.R. Noble, and T.W. Norton. 1992. A metapopulation simulation model for assessing the likelihood of plant and animal extinctions. Mathematical Computer Simulations 33:367-372.

Ralls, K., J.D. Ballou, and A. Templeton. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology 2:185-193.

Richter-Dyn, N. and N.S. Goel. 1972. On the extinction of a colonizing species. Theoretical Population Biology 3:406-433.

Robertson, A. 1960. A theory of limits in artificial selection. Proceedings of the Royal Society of London, Series B 153:234-239.

Rockwell, R.F. and G.F. Barrowclough. 1995. Effective population size and lifetime reproductive success. Conservation Biology 9:1225-1233.

Roelke, M.E., J.S. Martenson, and S.J. O'Brien. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Current Biology 3:340-350.

Ruckstuhl, K.E. 2000. Updated status report on wood bison *(Bison bison athabascae)* in Canada. June 18, 2000, Final report submitted to the Committee on the Status of Endangered Wildlife in Canada. 49 pp.

Ryder, O.A. and R.C. Fleischer. 1996. Genetic research and its application in zoos. Pages 255-262 *in* Kleiman, D.G., M.E. Allen, K.V. Thompson, S. Lumpkin, eds. Wild mammals in captivity: principles and techniques. University of Chicago Press, Chicago, IL.

Saccheri, I, M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491-493.

Shaffer, M.L. 1981. Minimum population sizes for species conservation. BioScience 31:131-134.

Shaffer, M.L. 1987. Minimum viable populations: coping with uncertainty. Pages 69-86 *in* Soulé, M.E., ed. Viable populations for conservation. Cambridge University Press, Cambridge, UK.

Soper, J.D. 1941. History, range and home life of the northern bison. Ecological Monographs 11:347-412.

Soulé, M., M. Gilpin, W. Conway, and T. Foose. 1986. The millenium ark: how long a voyage, how many staterooms, how many passengers? Zoo Biology 5:101-113.

Stephenson, R.O., S.C. Gerlach, R.D. Guthrie, C.R. Harington, R.O. Mills, and G. Hare. 2001. Wood bison in late Holocene Alaska and adjacent Canada: paleontological, archeological and historical records. Pages 125-159 *in* Gerlach, S.C. and Murray, M.S., eds. People and wildlife in northern North America: essays in honor of R. Dale Guthrie. British Archeological Reports, International Series. University of Alaska, Fairbanks, AK.

Swain, D.P. and B.E. Riddell. 1990. Variation in agonistic behavior between newly emerged juveniles from hatchery and wild populations of coho salmon *Oncorhyncus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences 47:566-571.

Templeton, A.R. and B. Read. 1984. Factors eliminating inbreeding depression in a captive herd of Speke's gazelle (*Gazella spekei*). Zoo Biology 3:177-199.

Van Vuren, D. 1984. Abnormal dentition in the American bison *Bison bison*. Canadian Field-Naturalist 98:366-367.

van Zyll de Jong, C.G. 1986. A systematic study of recent bison, with particular consideration of the wood bison (*Bison bison athabascae* Rhoads 1898). National Museum of Natural Sciences, Publication in Natural Sciences no. 6. Ottawa, ON. 69 pp.

van Zyll de Jong, C.G., C. Gates, H. Reynolds, and W. Olson. 1995. Phenotypic variation in remnant populations of North American bison. Journal of Mammalogy 76:391-405.

Vucetich, J.A. and T.A. Waite. 1998. Number of censuses required for demographic estimation of effective population size. Conservation Biology 12:1023-1030.

Wang, J. 2001. Optimal marker-assisted selection to increase the effective size of small populations. Genetics 157:867-874.

Wang, J., W.G. Hill, D. Charlesworth, and B. Charlesworth. 1999. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. Genetical Research. 74: 165-178.

Ward, T.J., J. Bielawski, S.K. Davis, J.W. Templeton, and J.N. Derr. 1999. Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap. Animal Conservation 2:51-57.

Wilson, G.A. 2001. Population genetic studies of wood and plains bison populations. Unpublished PhD Thesis, University of Alberta, Edmonton, AB. 156 pp.

Wilson, G.A., J. Nishi, B. Elkin, T. Ellsworth, C.C. Gates, and C. Strobeck. Conservation of wood bison (*Bison bison athabascae*) in Canada: a genetic perspective. (in prep.)

Wilson, G.A., W. Olson, and C. Strobeck. (2002). Reproductive success in wood bison (*Bison bison athabascae*), a polygynous mammal, established using molecular techniques. Canadian Journal of Zoology. (submitted)

Wilson, G.A. and C. Strobeck. 1999. Genetic variation within and relatedness among wood and plains bison populations. Genome 42:483-496.

Wood Bison Recovery Team. 1987. Status report on endangered wildlife in Canada 1987: Wood bison. Canadian Wildlife Service, Ottawa, ON. 87 pp.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-159.

Wright, S. 1969. Evolution and genetics of populations. Volume 2: the theory of gene frequencies. University of Chicago Press, Chicago, IL. 512 pp.

Wright, S. 1977. Evolution and the genetics of populations. Volume 3: experimental results and evolutionary deductions. University of Chicago Press, Chicago, IL. 614 pp.